MUSSEL BIOADHESIVE

BACKGROUND OF THE INVENTION

[FIELD OF THE INVENTION]

The present invention relates to a bio-adhesive derived from mussel, and more particularly to a novel *Mytilus galloprovincialis* foot protein-5 (MGFP-5) and a recombinant protein that is a hybrid of MGFP-5 and foot protein-1(FP-1).

[BACKGROUND OF THE INVENTION]

Mussels produce and secrete specialized water-resistant bioadhesives, and have been studied as a potential source of water-resistant bioadhesives. They adhere tightly to surfaces underwater using the byssus secreted from the foot of the mussel. At the end of each thread is an adhesive plaque containing a water-resistant glue that enables the plaque to anchor to wet solid surfaces (Waite, J. H., *Biology Review.* 58:209-231(1983). This strong and water-insoluble adhesion has attracted interest for potential use in biotechnological applications. Mussel adhesive proteins can also be used as medical adhesives as they are non-toxic to the human body and do not impose immunogenicity (Dove et al., *Journal of American Dental Association.* 112:879 (1986)). Moreover, their biodegradable properties make them environmentally friendly.

The byssus can be divided into distal and proximal parts. The proximal part is connected to the stem gland of the mussel foot, while the distal part is connected to the adhesive plaques. The adhesive plaque is composed of five distinct types of proteins: foot protein type 1 (FP-1) to type 5 (FP-5) (Deming, T. J., *Current Opinion*

in Chemical Biology. 3:100-105 (1999)).

All of the mussel adhesive proteins contain high ratios of 3,4-dihydroxyphenyl-L-alanine (DOPA), which is derived from hydroxylation of tyrosine residues (Waite, J. H., *Biology Review.* 58:209-231 (1983)). The adhesive proteins closest to the adhesion interface have the highest proportion of DOPA residues (Waite, J. H., *Integr. Comp. Biol.* 42:1172-1180 (2002)). In contrast, mussel adhesive protein analogs lacking DOPA show greatly reduced adhesion abilities (Yu et al., *Journal of American Chemical Society.* 121:5825-5826 (1999)). Indeed, a biochemical study showed that DOPA residues can enable mussel adhesive protein molecules to cross-link with each other via oxidative conversion to o-quinone. Thus, the DOPA content of a mussel adhesive protein appears to be specifically related to its adhesive properties.

Currently Cell-Tak, a naturally extracted mussel adhesive protein product, is commercially available. This adhesive is mainly composed of FP-1 and FP-2 type proteins, with a minor portion of FP-3. However, the natural extraction process is labor-intensive and inefficient, requiring around 10,000 mussels for 1 mg of protein (Morgan, D., *The Scientist.* 4:1-6 (1990)).

Therefore, researchers have sought to produce recombinant mussel adhesive proteins, for example FP-1, in expression systems such as *Escherichia coli* and yeast. However, these previous studies failed to express functional and economical mussel adhesive proteins due to a number of complications, including a highly biased amino acid composition (5 amino acid types comprise ~89% of the total amino acids in FP-1), different codon usage preferences between mussel and

other expression systems (tRNA utilization problems) and low protein yields (US Patent No. 5242808, Filpula et al., *Biotechnol. Prog.* 6:171-177 (1990), Salerno et al., *Applied Microbiology and Biotechnology* 58:209-214 (1993), Kitamura et al., Journal of Polymer Science Part A: Polymer Chemistry, 37:729-736 (1999)).

SUMMARY OF THE INVENTION

An objective of the present invention is to provide a novel adhesive protein gene from mussel and to overcome the aforementioned problems in the prior art.

Another objective of the present invention is to provide a novel adhesive protein from mussel.

Another objective of the present invention is to provide a method for mass-producing a mussel adhesive protein in a biologically active form.

Another objective of the present invention is to provide a recombinant adhesive protein that is a fusion of two or more adhesive proteins from mussel.

Another objective of the present invention is to provide an adhesive that contains a novel adhesive protein as an active component.

The present invention provides a novel adhesive protein extracted from *Mytilus galloprovincialis* and a polynucleotide encoding the protein. The above adhesive protein preferably comprises the amino acid sequence shown in SEQ ID NO: 6. An example of the above polynucleotide is the nucleotide sequence shown in SEQ ID NO: 5.

The present invention also provides a recombinant adhesive protein where some amino acid sequences from FP-1 is attached to the amino- and/or carboxy-

termini of a mussel adhesive protein, and a polynucleotide encoding the recombinant adhesive protein. An example of the recombinant adhesive protein is an amino acid sequence selected from the group consisting of the amino acid sequence shown in SEQ ID NO: 10, the amino acid sequence shown in SEQ ID NO: 12, the amino acid sequence shown in SEQ ID NO: 14, the amino acid sequence shown in SEQ ID NO: 18, the amino acid sequence shown in SEQ ID NO: 20, and the amino acid sequence shown in SEQ ID NO: 22. An example of the nucleotide sequence encoding the recombinant adhesive protein is the nucleotide sequence shown in SEQ ID NO: 9, the nucleotide sequence shown in SEQ ID NO: 11, the nucleotide sequence shown in SEQ ID NO: 13, the nucleotide sequence shown in SEQ ID NO: 17, the nucleotide sequence shown in SEQ ID NO: 19, and the nucleotide sequence shown in SEQ ID NO: 21.

The present invention also provides a vector which contains operably a nucleotide sequence encoding an adhesive protein.

The present invention also provides a transformant which contains operably a nucleotide sequence encoding an adhesive protein.

The present invention also provides a method of producing an adhesive protein which comprises the steps of:

(a) constructing a vector which contains operably a nucleotide sequence encoding an adhesive protein;

(b) constructing a transformant by transforming a host cell with the vector; and

(c) producing a recombinant adhesive protein by culturing the

transformant.

The present invention also provides a method of purifying an adhesive protein which comprises the steps of:

(a) lysing the transformants, and then centrifuging it to separate the supernatant and the pellet;

(b) making a suspension by adding an acidic organic solvent to the pellet; and

(c) centrifuging the suspension to separate the supernatant.

The present invention also provides an adhesive containing an adhesive protein as the active component.

The present invention also provides a method of adjusting the adhesive property of an adhesive comprising controlling the concentration of an adhesive protein which is an active component of the aforementioned adhesive, or treating the adhesive with one or more material selected from the group consisting of oxidant, filler and surfactant.

The present invention also provides a coating material containing an adhesive protein as an active component.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a picture of the electrophoresis of MGFP-5 cDNA fragments obtained by RT-PCR with RNA extracted from *Mytilus galloprovincialis* as the template.

Fig. 2 shows the procedure for inserting MGFP-5 cDNA into a pTrcHis

vector to construct a pMDG05 vector.

Fig. 3 shows the FP-1 variant (referred to as "6xAKPSYPPTYK" from hereon) where the peptide AKPSYPPTYK is tandemly attached 6 times, and the 4 oligomers KD-1, KD-2, KD-3, and KD-4 that were used in its synthesis.

Fig. 4 shows vector diagrams of various combinations that can simultaneously express the 6xAKPSYPPTYK and the MGFP-5 gene.

Fig. 5 is a diagram showing the procedure for constructing the pMDG051 vector for making the recombinant MGFP-51 nucleotide sequence.

Fig. 6 is a diagram showing the procedure for constructing the pMDG150 vector for making the recombinant MGFP-15 nucleotide sequence.

Fig. 7 is a diagram showing the procedure for constructing the pMDG151 vector for making the recombinant MGFP-151 nucleotide sequence.

Fig. 8 is a diagram showing the procedure for constructing the pENG151 vector for making the recombinant MGFP-151 nucleotide sequence.

Fig. 9 is a photograph of the electrophoresis of each culture material of *E. coli* BL21/pMDG05 and *E. coli* BL21/pTrcHis (*E. coli* BL21/pTrcHisA transformed with pTrcHisA)

Fig. 10 is a Western blot photograph of an SDS-PAGE of the whole cell pellet (WC), soluble upper fraction (I), and insoluble cell debris fraction (IS) isolated from *E. coli* BL21/pMDG05 culture solution, and negative control (N).

Fig. 11 is a photograph of silver-stained SDS-PAGE of affinity chromatography fractions for purification of recombinant MGFP-5 protein from *E. coli* BL21/pMDG05.

Fig. 12 is a mass spectrometry result of purified recombinant MGFP-5 protein.

Fig. 13 shows (A) Coomassie blue-stained SDS-PAGE and (B) Western blot analyses of recombinant MGFP-51 protein from *E. coli* BL21/pMDG051.

Fig. 14 shows (A) SDS-PAGE and (B) Western blot analyses of recombinant MGFP-15 protein from *E. coli* BL21/pMDG150.

Fig. 15 shows (A) SDS-PAGE and (B) Western blot analyses of recombinant MGFP-151 protein from *E. coli* BL21/pMDG151.

Fig. 16 shows the recovery rates of recombinant MGFP-151 protein expressed from *E. coli* BL21/pMDG151 according to the concentration of acetic acid solution.

Fig. 17 shows (A) SDS-PAGE and (B) Western blot analyses of chromatographic fractions of recombinant MGFP-151 protein.

Fig. 18 shows the results of coating slide glass, poly(methyl methacrylate) plate, and aluminum plate with recombinant MGFP-5 and recombinant MGFP-151 proteins after the tyrosine residues are modified to DOPA.

Fig. 19 shows the QCM analysis results of BSA, Cell-Tak, recombinant MGFP-5 protein, and recombinant MGFP-151 protein after treating the tyrosines.

Fig. 20 is a diagrm of the measurement method of the adhesion force of recombinant adhesive proteins.

Fig. 21 shows the adhesion force of recombinant MGFP-1 and recombinant MGFP-5 proteins where the tyrosine residues have been modified.

Fig. 22 is a measurement of cell-adhesion property of recombinant

adhesive proteins.

Fig. 23 is a measurement of cell-adhesion property of recombinant adhesive proteins with insect *Drosophila* S2 cells.

Figs. 24A to C are photographs of a substrate surface coated with recombinant MGFP-5 and recombinant MGFP-151 proteins. A is a 2500X enlargement, B is a 10000x enlargement, and C is a 35000x enlargement.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The inventors of the present invention have obtained a novel adhesion protein and its encoding gene from a type of mussel, *Mytilus galloprovincialis,* and they have established a system for the production of an adhesion protein that is translated from it. They have also established a recombinant adhesion protein that is a fusion of two or more mussel adhesion proteins, and a system for its production.

The adhesive protein of the present invention has the characteristic of attaching to a wide variety of substrates such as glass, metal, polymer resin, plastic or biological cell membranes such as prokaryotic membranes, eukaryotic membranes, and plant cell walls and lipids.

The adhesive protein of the present invention has at least 50 % homology with the amino acid sequence shown in SEQ ID NO: 6, preferably 80 %, more preferably 90 %, and most preferably at least 95 % homology, and at the same time can include amino acid sequences that have adhesive property, for example adhesive property that is similar to the amino acid sequence shown in SEQ ID NO: 6, or amino acid sequences that have 70 to 200 % of the adhesive activity of the above.

For example, there is a protein that contains the amino acid sequence shown in SEQ ID NO: 6. An adhesive protein that contains the amino acid sequence as shown in the above SEQ ID NO: 6 is referred to as "MGFP-5" (*Mytilus galloprovincialis* foot protein type 5) from hereon.

A nucleotide encoding MGFP-5 can be expressed as a variety of nucleotide sequences depending on the amino acid codon usage, such as the nucleotide sequences shown in SEQ ID NO: 5 and SEQ ID NO: 15.

Also, the adhesive protein of the present invention can further contain a peptide at the amino- and/or carboxy-termini in order to improve the physicochemical properties of the adhesive protein. The above peptide may be added for the purpose of improving for example, the solubility, adhesion force, degree of crosslinking, and the degree of expression, purification, and recovery of protein. For example, the above peptide can be a general reporter protein such as GST or a histidine tag for the purpose of improving the purification.

An example of a form where a peptide is further included for the purpose of purifying an adhesive protein is a protein containing the amino acid sequence shown in SEQ ID NO: 16.

The above peptide preferably contains an amino acid sequence derived from an adhesive protein, and more preferably contains an amino acid sequence derived from a mussel adhesive protein. An example of the peptide is the amino acid sequence shown in SEQ ID NO: 25 is repeated 1 to 10 times in tandem. In an embodiment of the present invention, a SEQ ID NO: 8 was constructed in which the amino acid sequence shown in SEQ ID NO: 25 is repeated 6 times in tandem, and

attached to the amino- and/or carboxy-termini of the adhesive protein in the present invention. The amino acid sequence shown in the SEQ ID NO: 25 is a part of the sequence of the FP-1 protein.

Examples of recombinant adhesive proteins where the sequence shown in SEQ ID NO: 25 is additionally attached, are amino acid sequences shown in SEQ ID Nos: 10, 12, and 14. The SEQ ID NO: 10 is where the sequence shown in SEQ ID NO: 25 is repeated 6 times in tandem and attached to the amino-terminus of the amino acid sequence shown in SEQ ID NO: 6. SEQ ID NO: 12 is where the sequence shown in SEQ ID NO: 25 is repeated 6 times in tandem and attached to the carboxy-terminus of the amino acid sequence shown in SEQ ID NO: 6. SEQ ID NO: 14 is where the sequence shown in SEQ ID NO: 25 is repeated 6 times in tandem and attached to the amino- and carboxy-termini of the amino acid sequence shown in SEQ ID NO: 6.

Furthermore, recombinant adhesive proteins containing the amino acid sequences shown in SEQ ID Nos: 10, 12, or 14 can additionally contain a peptide which has a purpose of facilitating purification. The peptide may be located on the amino- and/or the carboxy-termini of a recombinant adhesive protein, and examples of the peptide are GST and histidine tag. Recombinant adhesive proteins containing amino acid sequences as shown in SEQ ID Nos: 18, 20, or 22 are forms where a histidine tag is attached to the amino-terminus of a protein containing the amino acid sequences shown in SEQ ID Nos: 10, 12, or 14 respectively.

The adhesive protein of the present invention can further contain a peptide of 1 to 10 amino acids that are additionally inserted during cloning of the adhesive

protein, at the amino-terminus, carboxy-terminus, or another kind of connective region of proteins.

The adhesive protein and recombinant adhesive protein of the present invention can be inserted into commonly used expression vectors constructed for expressing exogenous genes, and mass-produced through genetic engineering methods. The above vector may be selected according to the type and characteristics of the host cell used in the production of protein, or it may be newly constructed. Transforming the vector into the host cell and producing the recombinant protein from the transformant can easily be carried out through ordinarily employed methods. Selecting, constructing, transforming the vector and expressing the recombinant protein can be easily carried out by an ordinary person skilled in the art of the present invention, and partial variations in the ordinarily employed methods are also included in the present invention.

The sequence encoding an adhesive protein that is inserted into the vector is a sequence encoding an adhesive protein or a recombinant adhesive protein of the present invention, and is preferably selected from the group consisting of a nucleic acid encoding a protein that has at least 50 % homology, preferably 80 %, more preferably 90 %, and most preferably at least 95 % homology with the amino acid sequence shown in SEQ ID NOs: 6, 10, 12, or 14, a nucleic acid encoding a protein that has at least 50 % homology, preferably 80 %, more preferably 90 %, and most preferably at least 95 % homology with the amino acid sequence shown in SEQ ID NO: 6, 10, 12, or 14, where at least one sequence selected from the group consisting of SEQ ID NO:s 26 to 31 is tandemly repeated 1 to 10 times at the 5' and/or 3' ends

of the nucleic acid, and the amino acid sequence shown in SEQ ID NO: 6, 10, 12, or 14, where 6 histidine residues are additionally attached at the amino-terminus. More preferably, a polynucleotide containing a sequence selected from the group consisting of SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, 19 and 21 can be inserted into the vector.

In an embodiment of the present invention, MGFP-5 sequence was cloned into a pGEM-T vector, and the sequence shown in SEQ ID NO: 7 (6xAKPSYPPTYK which is 6 tandem repeats of the amino acid sequence shown in SEQ ID NO: 25) was cloned into pUC18. Afterwards, the MGFP-5 sequence was cloned into a pTrcHisA vector to construct a pMDG05 vector (Fig. 4). Furthermore, in order to construct a vector that expresses a recombinant protein having a structure shown in Table 1 below, the sequences of MGFP-5 and SEQ ID NO: 7 were cloned into a pTrcHisA vector to construct pMDG150, pMDG051 and pMDG151 vectors (Fig. 7 to 9).

(Table 1)

Hybrid adhesive protein	Structure (5' to 3')	Vector
MGFP-15	6 x AKPSYPPTYK - MGFP-5	pMDG150
MGFP-51	MGFP-5 – 6 x AKPSYPPTYK	pMDG051
MGFP-151	6 x AKPSYPPTYK - MGFP-5 – 6 x AKPSYPPTYK	pMDG151 pENG151

The above pTrcHisA vector is a widely known vector which contains a *trc*promoter, which allows expression of exogenous protein by induction using IPTG(isopropylthio-β-D-galactoside), and which has 6 histidine sequences for protein purification by affinity chromatography at the 5' end of the exogenous gene in order to facilitate protein purification. In the present invention, the pMDG05

vector was deposited at the Korean Collection for Type Cultures (KCTC) at the Biological Resource Center of Korea located at Eouen-dong, Yuseong-gu, Daejon, Republic of Korea as of June 20, 2002, and received an accession number of KCTC 10291BP. The pENG151 vector was deposited as of January 19, 2005 and given an accession number of KCTC 10766BP.

The expression vector for the adhesive protein and recombinant adhesive protein can be transformed into a host cell selected from the group consisting of prokaryotes, eukaryotes, and eukaryote-derived cells, in order to construct a transformant. The prokaryote is selected from the group consisting of *E. coli* and *Bacillus*, the eukaryote is selected from the group consisting of yeast, insects, animals, and plants, and the eukaryote-derived cells are plant cells, insect cells, and plants, but is not limited thereto.

As an embodiment, pMDG05, pMDG150, pMDG051 and pMDG151 vectors were each transformed into *E. coli BL21*, to construct *E. coli BL21*/pMDG05, *E. coli BL21*/pMDG150, *E. coli BL21*/pMDG051 and *E. coli BL21*/pMDG151. The aforementioned 4 types of transformants can be cultured in typical LB media, and IPTG can be added to induce protein expression. The preferred method of expression of recombinant protein is to culture in LB media (5 g/liter yeast extract, 10 g/liter Tryptone, 10 g/liter NaCl), and adding 0.1 to 10 mM of IPTG when the *optical density* of the culture solution is 0.6 to 0.9 at 600 nm, then culturing for 2 to 12 hours.

The recombinant protein expressed in the above method is expressed in a water-soluble and/or insoluble form within the transformant, so the isolation and

purification depends on how it is expressed. When it is expressed in a water-soluble form, the recombinant protein can be purified by running the lysed cell supernatant through a chromatography column filled with an affinity resin such as a nickel resin. When it is expressed in a water-insoluble form, the recombinant protein can be purified by suspending the lysed cell pellet in an acidic organic solvent, preferably an organic solvent with a pH of 3 to 6, then centrifuging the suspension to isolate the upper layer. Examples of the acidic organic solvent are acetic acid, citric acid, and lactic acid, but is not limited thereto. The acetic acid used can be 5 to 30 (v/v) %, and preferably the cell pellet is dissolved in 20 to 30 (v/v) % acetic acid solution. The upper layer obtained through treatment with acidic organic solvent can further undergo gel filtration chromatography to further purify the recombinant protein.

Through the method of the present invention, 2-3 mg/L of the recombinant adhesive protein MGFP-5 of at least 95% purity can be obtained, and around 5 mg/L of MGFP-151 of at least 95% purity can be obtained. While MGFP-5 and MGFP-151 display similar levels of adhesion force, the solubility of MGFP-151 is significantly higher compared to MGFP-5, and thus MGFP-151 is easier to obtain in a concentrated form. In particular, MGFP-5 dissolves in a 5 % acetic acid solution to a concentration of around 1 mg/mL, while MGFP-151 dissolves in water to a concentration of around 110 mg/mL, and dissolves in a 5 % acetic acid solution to a concentration of around 220 mg/mL. The solubility of an adhesive protein is directly related to its ability to stay in highly concentrated forms, so the higher the solubility, the easier it is to make highly concentrated forms with high potential for industrial application. In this respect, it can be said that the adhesive protein MGFP-151 is

more useful than MGFP-5.

The adhesive protein and the recombinant adhesive protein obtained through its expression in the present invention have adhesive activity and can be used as adhesives. The adhesive activity was confirmed through the experiment of modifying the tyrosine residues in the protein to 3,4-dihydroxyphenyl-L-alanine (DOPA). Thus, the adhesive protein of the present invention can not only be used as an adhesive for a wide variety of substrates, but also be used as a bioadhesive since it is harmless to the human body.

The present invention also provides an adhesive that contains adhesive protein as an active component. The adhesive protein can be a form where 5 to 100 % of its tyrosine residues are modified to DOPA, and the adhesive can additionally contain a substance that modifies the tyrosine residues in the protein to DOPA. A typical example of the above substance is tyrosinase, but is not limited thereto.

The above adhesive can further contain 0.5 to 90 % by weight of an excipient that is generally contained in bioadhesives or is pharmaceutically acceptable. Examples of excipients include surfactants, oxidants, and fillers, but are not limited thereto (see: US Pat. Application Publication No. 2003-65060 and US Pat. No. 5,015,677). The surfactant can be cationic, anionic, non-ionic, or amphoteric, where examples are sodium dodecylsulfate and sodium dodecylbenzensulfonate. The oxidant can be selected from the group consisting of tyrosinase, catechol oxidase, glutaraldehyde, formaldehyde, bis(sulfosuccinimidyl) suberate, 3,3'-Dithiobis(sulfosuccinimidyl propionate), O_2, Fe^{3+}, H_2O_2 and IO_4^-(see:

Macromolecules 1998, 31, 4739-4745), and the filler can be selected from the group consisting of collagen, hyaluronic acid, condroitan sulfate, elastine, laminin, caseine, hydroxyapatite, albumin, fibronectin, and hybrin.

The adhesive of the present invention can be used to adhere or fix glass, plastic, polymer resin, or biological specimen, and the detailed mode and amount of usage, formulation and other such matters may follow Cell-Tak (BD Biosciences, Two Oak Park, Bedford, MA, USA) which is currently available commercially. For example, the adhesive of the present invention can be a soluble, water-soluble, or insoluble formulation, and can be used in the amount of 0.01 to 100 ug/cm^2 for a substrate but is not limited thereto. Furthermore, the mode of use follows the general mode of adhesive use, and the typical mode is coating.

The aforementioned biological specimen refers to any animal or plant categorized as a biological organism and any part derived from such animal or plant. For example, it refers to cells, tissues, organs, RNA, DNA, protein, peptide, polynucleotide, hormones, and compounds, but is not limited thereto.

Examples of application of the adhesive of the present invention are as follows, but not limited thereto: (1) adhesion of substrates under water (fresh or salt water); (2) orthopedic treatments such as treatment of bone, ligament, tendon, meniscus, and muscle, and implant of artificial materials; (3) treatment of perforations, lacerations, and cuts, and ophthalmic attachments such as corneal implants and artificial corneal implants; (4) dental attachments such as holding retainers, bridges, or crowns in place, securing loose teeth, repairing broken teeth, and holding fillers in place; (5) surgical treatments such as attachment of blood

vessels , attachment of cellular tissue, artificial material implants, and closure of wounds; (6) plant attachments such as bonding of transplanted parts and wound healing; (7) drugs, hormones, biological factors, medications, physiological or metabolic monitoring equipment, antibiotics, and cell transplant (see: US 5,015,677).

The present invention also provides a method of adjusting the adhesion force of the above adhesive by treating with a substance selected from the group consisting of surfactant, oxidant, and filler, or controlling the concentration of the adhesive protein which is an active component of the adhesive (see: US Pat No.5,015,677). The surfactant, oxidant, and filler are the same as was described above.

The present invention also provides a coating agent which contains the above adhesive protein as an active component. Since the adhesive protein of the present invention has the characteristic of adhering to glass, plastic, polymer resin, or biological specimen, it can not only be used as a coating agent for these substrates, but also coat the surface of substrates that are used underwater to prevent oxidation of the substrates, since the adhesive protein is water-resistant and water-repellent. An example of application of the coating agent is to coat the motor propeller of ships to prevent corrosion, but is not limited thereto. The above coating agent may consist solely of an adhesion protein, but can additionally contain commonly known adhesives, adhesive proteins other than the adhesive proteins of the present invention, resin contained in commonly known coating agents, organic solvents, surfactants, anticorrosive agents, or pigments. The content of the additional components may be appropriately adjusted within the commonly accepted range depending on the kind of

component and formulation of the coating agent. Where an additional component is included, the adhesive protein as an active component is contained in the coating agent at a level that maintains the adhesive activity, and can for example be contained in the coating agent at 0.1 to 80 % by weight.

The coating agent of the present invention can be manufactured in the form of cream, aerosol (spray), solid, liquid, or emulsion, but is not limited to these formulations.

Embodiments of the present invention are described below. The following embodiments are merely illustrative of the present invention and the present invention is not limited to the following embodiments.

In the following, the mussel used for cloning the MGFP-5 gene was *Mytilus galloprovincialis.*

Example 1: Cloning of the MGFP-5 gene

In order to clone MGFP-5, the primer shown in SEQ ID NO: 1 (5'-ggcctgcagcagttctgaagaatacaaggg-3) and the primer shown in SEQ ID NO: 2 (gtagatctatacgccggaccagtgaacag) were each synthesized. PCR was run 30 times using the mussel cDNA library, and a PCR product of 243 bp was obtained (Fig. 1). The above PCR product was cloned in a pGEM-T vector (Promega).

To obtain the upstream signal sequence of MGFP-5, nested PCR was executed using mussel (*M. galloprovincialis*) cDNA library. The primers used were a T3 promoter primer of the ZAP vector (SEQ ID NO: 32) and the primer shown in SEQ ID NO: 3 (5'-cttgtattttccgctgttttt-3'). An amplification product of around 300 bp

was obtained through the PCR and it was cloned into a pGEM-T vector.

To obtain the C-terminal poly-A tail region of MGFP-5, nested PCR was performed using SEQ ID NO: 4 (5′-aaaaacagcggaaaatacaag-3′) and T7 promoter primer (SEQ ID NO: 33). The amplification product of 350 bp was obtained and cloned into a pGEM-T vector.

The MGFP-5 cDNA nucleotide sequence obtained from the above was analyzed, and the MGFP-5 nucleotide sequence excluding the secretion signal sequence is shown in SEQ ID NO: 5, and the amino acid sequence encoded therein is shown in SEQ ID NO: 6.

Example 2: Construction of the vector for genetically engineered production of MGFP-5

The MGFP-5 cDNA in the pGEM-T vector was isolated by using the restriction enzyme sites *Pst*I and *EcoR*I, then inserted into a pTrcHis A vector (Invitrogen, USA) that was cleaved with *Pst*I and *EcoR*I restriction enzymes to construct pMDG05(4630bp). The pMDG05 vector was deposited at the Korean Collection for Type Cultures (KCTC) at the Biological Resource Center of Korea located at Eoeun-dong, Yuseong-gu, Daejon, Republic of Korea as of June 20, 2002, and received a accession number of KCTC 10291BP.

The pMDG05 vector contains a *trc* promoter for expression in *E. coli*, and allows induction of expression using IPTG (Sigma, USA). It also has 6 histidine residues at the 5' end of the MGFP-5 gene for protein isolation and purification by affinity chromatography.

Example 3: Construction of the peptide (6xAKPSYPPTYK) derived from FP-1

From the amino acid sequence of FP-1 (Genbank No.Q27409 or S23760), an FP-1 derivative as shown in SEQ ID NO: 25 where the peptide "AKPSYPPTYK" is tandemly repeated 6 times (referred to as "6xAKPSYPPTYK") was constructed.

That is, KD-1 to KD-4 described in Fig. 3 was each synthesized, and then annealed, to synthesize 6xAKPSYPPTYK in SEQ ID NO: 8 encoding the FP-1 variant in SEQ ID NO: 7. Additionally, at the 5' end of the 6xAKPSYPPTYK, *EdoR*I and *Nhe*I restriction enzyme sites in the direction of 5' to 3' were placed, and a *BamH*I restriction enzyme site was placed at the 3' end (Fig. 3). The 6xAKPSYPPTYK was inserted into a pUC18 vector using the *Nhe*I and *BamH*I restriction enzyme sites to construct the pAD501 vector (M. Kitamura, 1999, Journal of Polymer Science Part A: Polymer Chemistry 37, 729-736).

In Fig. 3, the "ACTAT" located at the 5' side of the *BamH*I site in the polynucleotide was inserted to preserve the ORF.

Example 4: Construction of recombinant hybrids of FP-1 and MGFP-5

From hereon, MGFP-5 is referred to as "MGFP-5", a hybrid where 6xAKPSYPPTYK of Example 3 is attached to the N-terminus of MGFP-5 is referred to as "MGFP-15", a hybrid where 6xAKPSYPPTYK is attached to the C-terminus of MGFP-5 is referred to as "MGFP-51", and a hybrid where 6xAKPSYPPTYK is attached to both the N- and C-termini of MGFP-5 is referred to as "MGFP-151" (see

Table 1 above).

The hybrids MGFP-15 shown in SEQ ID NO: 10, MGFP-51 shown in SEQ ID NO: 12, and MGFP-151 shown in SEQ ID NO: 14 were constructed, and they include histidine tags (6x His) and other amino acid residues at the 5' end and amino acid residues between the 6xAKPSYPPTYK and MGFP-5 due to the design of the experiment.

In order to express each of the hybrids MGFP-5, MGFP-15, MGFP-51 and MGFP-151, the structures shown in SEQ ID Nos: 15, 17, 19, and 21 were inserted into a vector to respectively construct pMDG05, pMDG150, pMDG051, and pMDG151 of Fig. 4. In the above 4 vectors, expression is controlled by a *trc* promoter that is inducible by IPTG (Sigma, US), and there are 6 histidine residues at the 5' region and a translation termination codon (TAA) at the 3' end of each recombinant construct.

The method of construction for each vector is as follows.

PCR was executed on the nucleotide sequence of MGFP-5 within the pMDG05 vector of Example 2 with the primer set shown in SEQ ID NOs: 1 and 2, then cleaved with *Pst*I and *EcoR*I restriction enzymes, then inserted into a pTrcHis A vector (Invitrogen, USA) which was previously cleaved with the same enzymes, to construct pTEMP150(4630bp). Also, the 6xAKPSYPPTYK in the pAD501 vector of Example 2 was amplified with the primer set shown in SEQ ID NO: 23 (5'- GGT ACC CGA ATT CGA ATT CGC TAA ACC G-3') and 24 (5'-GGT CGA CTC AAG CTT ATC ATT TGT AAG TCG-3'), and cleaved with *EcoR*I and *Hind* III restriction enzymes. Then it was inserted into pTEMP150 which was previously cleaved with

*EcoR*I and *Hind* III, to construct pMDG051 (Fig. 5).

The 6xAKPSYPPTYK in the pAD501 vector was isolated by treating with *Nhe* I and *BamH* I restriction enzymes, and inserted into a pMDG05 vector which was treated with the same enzymes to construct pMDG150 (Fig. 6).

The 6xAKPSYPPTYK in the pAD501 vector was isolated by treating with *Nhe* I and *BamH* I restriction enzymes, and inserted into a pTrcHis A vector (Invitrogen, USA) which was treated with the same enzymes, to construct pTEMP1(4523bp). Then the nucleotide sequence of MGFP-5 within the pMDG05 vector was amplified with the primer set shown in SEQ ID NOs: 1 and 2, then cleaved with *Pst*I and *EcoR*I restriction enzymes, then inserted into pTEMP1 which was treated with the same enzymes, to construct pTEMP2(4741bp). Also, the 6xAKPSYPPTYK in the pAD501 vector was amplified with the primer set shown in SEQ ID NOs: 23 and 24, then cleaved with *EcoR*I and *Hind* III restriction enzymes. Then it was inserted into pTEMP2 which was cleaved with *EcoR*I and *Hind* III, to construct pMDG151 (4927bp) (Fig. 7).

Also, in order to express the MGFP-151 nucleotide at a high level, the MGFP-151 nucleotide was amplified from the pMDG151 vector with the primers shown in SEQ ID NO: 34 (5'- CCT AAC ATA TGG GGG TTC TCA TCA TC – 3') and SEQ ID NO: 35 (5'- ATC CGC CAA AAC AGC CAA GCT T – 3'). The amplified product was inserted into a pET 22b(+) vector (Novagen, EMB Bioscience, Inc. 441 Charmany Dr. Madison, WI 53719 USA) using *Nde* I and *Hind* III restriction enzymes, to construct a pENG151 vector (Fig. 8). The pENG151 vector was transformed into *E.coli,* and deposited at the Korean Collection for Type

Cultures (KCTC) at the Biological Resource Center of Korea as of January 19, 2005 and given an accessiont number of KCTC 10766BP.

Example 5: Construction of transformant producing MGFP-5 and hybrids

Competent cells of *E. coli* Top10 (*F-mcrA*(*mrr-hsdRMS-mcrBC*)Φ*80lacZΔM15 ΔlacX74 deoR recA1 araD139 Δ*(*ara-leu*)*7697 galU galK rpsL (Strr) endA1 nup*, Invitrogen) used for cloning, and *E. coli* BL21 (*F- ompT hsdSB* (*rB- mB-*) *gal dc*) used for protein expression were each prepared using $CaCl_2$ buffer. Transformation of each of the pMDG05, pMDG051, pMDG150 and pMDG151 vectors in Example 4 into the competent cells were achieved by a heat shock method (leaving for 2 minutes at 42 °C). Then through a selection process using ampicillin (Sigma) the transformants *E. coli* Top10/pMDG05, *E. coli* Top10/pMDG051, *E. coli* Top10/pMDG150, *E. coli* Top10/pMDG151, *E. coli* BL21/pMDG05, *E. coli* BL21/pMDG051, *E. coli* BL21/pMDG150 and *E. coli* BL21/pMDG151 were each obtained.

Example 6: Expression and purification of MGFP-5 from *E. coli* BL21/pMDG05

6-1. Culture of *E. coli* BL21/pMDG05

E. coli BL21/pMDG05 was cultured in LB media (5 g/liter yeast extract, 10 g/liter Tryptone and 10 g/liter NaCl), and IPTG was added to a final concentration of 1 mM when the optical density of the culture solution was 0.7 to 0.8 at 600 nm, to induce expression of recombinant adhesive protein MGFP-5. At this time, a culture

solution in 10 mL of LB media (with 500 μg of added ampicillin) cultured for 12 hours in a sterile 50 mL tube was inoculated into 100 mL of LB media contained in a 500 mL flask. The *E. coli* BL21/pMDG05 culture was centrifuged at 18,000 g for 4 to 10 minutes to obtain the cell pellet, and this was stored at -80 °C.

6-2. Confirmation of MGFP-5 expression

The cell pellet was resuspended in SDS-PAGE buffer (0.5 M Tris-HCl, pH 6.8, 10 % glycerol, 5 % SDS, 5 % β-mercaptoethanol, 0.25 % bromophenol blue) 100 μℓ, and denatured by boiling at 100 °C for 5 minutes. For SDS-PAGE analysis, the samples were electrphoresed on a 15% SDS-polyacylamide gel and then the protein bands detected using Coomassie blue staining (Bio-Rad) or silver staining (Bio-Rad, USA). For Western blot analysis, the samples were electrphoresed on a 15% SDS-polyacylamide gel and then transferred onto a nitrocellulose membrane at 15 V. The MGFP-5 protein transferred onto the nitrocellulose membrane was detected using a monoclonal anti-histidine ligand antibody (R&D Systems, USA) and a colorimetric reaction.

Fig. 9 is an electrophoretic image of the culture product of *E. coli* BL21/pMDG05 and *E. coli* BL21/pTrcHis (*E. coli* BL21/pTrcHisA transformed with pTrcHisA), where MW is a size marker, N is a control group, and WC is the culture product of *E. coli* BL21/pMDG05. Fig. 9 confirmed the expression of MGFP-5 protein in *E. coli* BL21/pMDG05.

To confirm the expressed form of recombinant MGFP-5 in the cell sample, SDS-PAGE and Western blot was carried out on each of the cell debris and soluble supernatant obtained from lysis of the cell pellet, and the cell pellet.

That is, *E. coli* BL21/pMDG05 cell pellets were resuspended in 5 ml Buffer B (8M urea, 10 mM Tris-Cl, 100 mM sodium phosphate, pH 8.0) per 1 gram cells, and were lysed by gentle shaking for 1 h at room temperature. The lysate was centrifuged at 14,000 rpm for 20 min to obtain the soluble supernatant and insoluble cell debris.

Fig. 10 is a Western blot image of the SDS-PAGE analysis of the cell pellet (WC), soluble supernatant (I), the insoluble cell debris (IS) isolated from the culture solution of *E. coli* BL21/pMDG05 and the negative control (N). Fig. 10 shows that the MGFP-5 protein is detected at high levels in the soluble supernatant fraction, indicating that it is expressed in a soluble form inside the cell.

6-3. Purification of recombinant MGFP-5 protein

In order to isolate and purify recombinant MGFP-5 which is expressed in a soluble form within *E. coli* BL21/pMDG05, affinity chromatography utilizing the histidine affinity ligand contained in the pMDG05 vector was carried out.

Immobilized metal affinity chromatography (IMAC) purification was performed using the Acta Prime Purification System (Amersham Biosciences) at room temperature at a 1 ml per min flow rate. 10 ml Ni-NTA™ Agarose (Qiagen) charged with 0.1 M $NiSO_4$ (Samchun Chemicals) was used as the resin, and separation was performed under denaturing conditions. After the column was filled with the resin, it was equilibrated with buffer (8M urea, 10 mM Tris-Cl, 100 mM sodium phosphate, pH 8.0). Then the soluble supernatant fraction was loaded onto the column, and then the column was eluted with Buffer A (8M urea, 10 mM Tris-Cl, 100 mM sodium phosphate, pH 6.3) and Buffer B (8M urea, 10 mM Tris-Cl, 100

mM sodium phosphate, pH 5.9). Recombinant MGFP-5 protein was eluted with elution buffer (8M urea, 10 mM Tris-Cl, 100 mM sodium phosphate, pH 4.5), and eluted fractions were all collected and dialyzed in 5% acetic acid at 4 °C (Spectra/Por® molecular porous membrane tubing, Spectrum Laboratories, USA).

Fig. 11 is an image of silver-stained SDS-PAGE of affinity chromatography fraction samples during the purification of recombinant MGFP-5 protein from *E. coli* BL21/pMDG05. M is a size marker, Lane 1 is the fraction that was adsorbed when the soluble supernatant fraction was loaded onto the column, Lane 2 is the eluted fraction obtained at the step of washing the column, and Lane 3 is the eluted fraction separated by the elution buffer. Fig. 11 shows that the recombinant is purified at a high level of purity.

6-4. Analysis of MGFP-5 recombinant protein

MALDI-TOF (Matrix-assisted laser desorption ionization with time-of-flight) mass spectrometry analysis was performed using a PerSeptive Voyager DE instrument (Perkin-Elmer).

Sinapinic acid in 30 % acetonitrile and 0.1 % trifluoroacetic acid was used as matrix solution. The recombinant MGFP-5 protein obtained from the above section 6-3 was diluted 1:25 with the matrix solution, then 1 μl was spotted onto gold plates and evaporated using a vacuum pump. Mass spectra were acquired in positive ion mode using an accelerating voltage of 25,000 V, grid voltage at 70 to 80 %, guide wire voltage at 0.3 %, delay time of 200 to 500 ns and N_2 laser power at 1600 to 1900 (arbitrary units). Internal calibration was performed using BSA with [M +

$H]^+$ at 66.431 and $[M + 2H]^{2+}$ at 33.216.

Fig. 12 is the result of mass spectrometry analysis of MGFP-5 recombinant protein.

Example 7: Expression and purification of MGFP-51 from *E. coli* BL21/pMDG051

E. coli BL21/pMDG051 was cultured and the cell pellet, cell debris and soluble supernatant fractions were each obtained in the same method as in Example 6. Afterwards, SDS-PAGE and Western blot was carried out on each of the above samples.

Fig. 13 shows photographs of SDS-PAGE analysis (A) and a Western blot analysis (B) of the expression of recombinant MGFP-51 protein from *E. coli* BL21/pMDG051. W is cell pellet, S is soluble supernatant fraction, and IS is insoluble cell debris. Fig. 13 shows that the recombinant MGFP-5 protein is expressed within the cell in both soluble and insoluble forms.

Example 8: Expression and purification of MGFP-15 from *E. coli* BL21/pMDG150

E. coli BL21/pMDG150 was cultured and the cell pellet, cell debris and soluble supernatant fractions were each obtained in the same method as in Example 6. Afterwards, SDS-PAGE and Western blot was carried out on each of the above samples.

Fig. 14 shows photographs of SDS-PAGE analysis (A) and a Western blot

analysis (B) of the expression of recombinant MGFP-15 protein from *E. coli* BL21/pMDG150. M is a size marker, W is cell pellet, S is soluble supernatant fraction, and IS is insoluble cell debris. Fig. 14 shows that the recombinant MGFP-15 protein is expressed within the cell in both soluble and insoluble forms.

Example 9: Expression and purification of MGFP-151 from *E. coli* BL21/pMDG151

9-1. Expression of MGFP-151

E. coli BL21/pMDG151 was cultured and the cell pellet, cell debris and soluble supernatant fractions were each obtained in the same method as in Example 6. Afterwards, SDS-PAGE and Western blot was carried out on each of the above samples.

Fig. 15 shows photographs of SDS-PAGE analysis (A) and a Western blot analysis (B) of the expression of MGFP-151 recombinant protein from *E. coli* BL21/pMDG151. W is cell pellet, S is soluble supernatant fraction, and IS is insoluble cell debris. Fig. 15 shows that the MGFP-151 recombinant protein is expressed within the cell in soluble and insoluble forms.

9-2. Purification I of recombinant MGFP-151 protein

Cell pellets were resuspended in 5 ml lysis buffer (10 mM Tris-Cl, 100 mM sodium phosphate, pH 8.0) per gram of cell pellet, then the cells were lysed at 20,000 PSI (Constant systems, Low March, UK). Cell lysates were centrifuged at 18,000 g and 4°C for 20 min to collect the cell debris. The cell debris was resuspended in 20 ml of 5, 10, 15, 20, 25, 30, 22, 24, 26 and 28 (v/v) % acetic acid

solutions per gram cell weight respectively and centrifuged under the same conditions. The supernatant was prepared into a chromatographic sample.

A 40 cm x 2.6 cm column was filled with Sephacryl S-300 HR (Pharmacia), and equilibrated with 5% acetic acid. Afterwards, 2 ml of sample was loaded, and then the sample was eluted with the concentrations of acetic acid that were used to resuspend each sample to collect the eluted fractions. The degree of purification of MGFP-151 in each concentration of acetic acid elution fraction was confirmed by SDS-polyacylamide gel electrophoresis and then staining by Coomassie blue (Fig. 16). Fig. 16 shows that recombinant MGFP-151 protein is eluted at an acetic acid concentration of 20 to 30 (v/v) %. In particular, it was confirmed that dilution in 25% acetic acid solution showed the best results with 74% purity and 45% yield.

9-3. Purification II of recombinant MGFP-151 protein

Cell pellets were resuspended in 5 ml lysis buffer (10 mM Tris-Cl, 100 mM sodium phosphate, pH 8.0) per gram of cell pellet, then the cells were lysed at 20,000 PSI (Constant systems, Low March, UK). Cell lysates were centrifuged at 18,000 g and 4°C for 20 min to collect the cell debris. The cell debris was resuspended in 20 ml of 25 % acetic acid solution per gram cell weight, and centrifuged under the same conditions. After the supernatant was freeze-dried, it was dissolved in 2 ml of 5% acetic acid and prepared into a chromatographic sample.

A 40 cm x 2.6 cm column was filled with Sephacryl S-300 HR (Pharmacia), and equilibrated with 5% acetic acid. Afterwards, 2 ml of sample was loaded, and the sample was eluted with 5 (v/v) % acetic acid and eluted fractions I and II were collected. The degree of purification of MGFP-151 in eluted fractions I

and II was confirmed by SDS-polyacylamide gel electrophoresis and then staining by Coomassie blue (Fig. 17). Fig. 17 shows photographs of SDS-PAGE analysis (A) and a Western blot analysis (B) of the chromatographic fractions of MGFP-151 recombinant protein. recombinant MGFP-151 protein with 95.8% purity could be isolated by chromatography.

The purified recombinant MGFP-151 protein was confirmed to be the same at the initially designed MGFP-151 peptide, through analysis with MALDI-TOF mass spectrometer.

Example 10: Modification of the adhesive protein's tyrosine residues

The MGFP-5, MGFP-51, MGFP-15 and MGFP-151 adhesive proteins purified in Examples 6 to 9 were each dissolved to a concentration of 1.44 mg/ml in 5% acetic acid buffer containing 25 mM ascorbic acid. Then after the addition of 50 ug/ml of tyrosinase, it was shaken for 6 hours at 25 °C. Through this process, the tyrosine residues of the adhesive proteins were modified to DOPA. Furthermore, bovine serum albumin (BSA) was used as negative control, and the commercial product Cell-Tak™(BD Bioscience, Two Oak Park, Belford, MA, USA) which consists of mussel adhesive proteins FP-1 and FP-2 was used as positive control.

Example 11: Verification of the ability of the recombinant MGFP-5 and MGFP-151 proteins to coat various surfaces

The abilities of recombinant MGFP-5 and MGFP-151 proteins to coat the surfaces of glass slide, poly(methyl methacrylate) plate, and aluminum plate were

measured. Each material surface was cleaned by washing with water several times and drying with nitrogen gas. A 5 μl drop of 1.44 mg/ml protein solution was spotted onto each surface and kept for 12 h at 25 °C. After drying, each surface was washed thoroughly with double distilled water for 2 h with shaking, and the water remaining on each surface was evaporated with a vacuum pump. After drying, the protein coated on the surface was visualized using Coomassie blue staining. This result is shown in Fig. 18.

Fig. 18 shows the result of coating the surfaces of glass slide, poly(methyl methacrylate) plate, and aluminum plate with recombinant MGFP-5 and MGFP-151 proteins after their tyrosine residues are modified to DOPA. The recombinant MGFP-5 and MGFP-151 proteins adhered to glass, poly(methyl methacrylate), and aluminum even before modification of their tyrosine residues, and the adhesion force was found to be much higher when the tyrosine residues are modified to DOPA.

Example 12: Measurement of adsorption of recombinant adhesive protein using QCM(Quartz Crystal Microbalance)

The quartz crystal used (Seiko EG & G) was a gold-coated AT-cut quartz 5 mm in diameter with a basic resonant frequency of 9 MHz. A 5 μl drop of a 1.44 mg/ml protein solution (BSA, Cell-Tak, recombinant MGFP-5 protein, and recombinant MGFP-151 protein) was each placed onto the gold surface of the quartz crystal and kept at 25 °C in a constant-temperature water bath for 1 hour. After taking it out of the water bath and drying, the gold surface was rinsed thoroughly in double distilled water for 1 h with shaking and the water remaining on the quartz crystal was

evaporated using a vacuum pump. Dried quartz crystal was connected to an EQCM controller (QCA917; Seiko EG & G) and variations in resonance frequency were measured. Since the resonance frequency of the quartz crystal decreases as a function of increase in the mass adsorbed on its surface (G. Sauerbrey, 1959, Z. Phys, 155, 206), the increase in mass was calculated by Equation 1 (M. Thomson, 1991, Analyst, 116, 881-889) with the value for change in resonance frequency.

(Equation 1)

$$\Delta mass = \frac{-\Delta freq \times A \times \sqrt{\mu_q \times \rho_q}}{2 \times F_q^{\,2}}$$

In the above Equation 1, Δmass is change in mass, Δfreq is change in resonance frequency, μ_q is AT-cut quartz crystal constant (2.947 x 1.011 g/cm/sec^2), P_q is the quartz crystal density (2.648 g/cm^2), F_q^2 is reference frequency (9.00 MHz), and A is quartz crystal surface area (0.196 cm^2).

Fig. 19 is the result of QCM analysis of BSA, Cell-Tak, recombinant MGFP-5 protein and recombinant MGFP-151 protein where the tyrosines were treated, showing the level of adsorption onto the gold surface as change in frequency. In Fig. 19, recombinant MGFP-151 protein modified with tyrosinase showed the greatest change in frequency, indicating the greatest mass adsorbing to the gold surface of the quartz crystal. Although Cell-Tak™ adsorbed in a greater amount compared to BSA, its adhesion force was much lower compared to that of recombinant MGFP-5 protein. The change in mass of recombinant MGFP-151 protein was 36 %, that of recombinant MGFP-5 protein was 23.2 %, and that of Cell-Tak was 10 % or less.

Example 13: Measurement of adhesion force of recombinant adhesion protein

using AFM(atomic force microscopy)

The force-distance curve was obtained using AFM (SPA400; Seiko Instruments), and AFM cantilevers were done according to the technique of Ducker et al. (W. Ducker, Nature, 1991, 353, 239-241) (Fig. 20). The cantilevers used for the present experiments were Olympus oxide-sharpened silicon nitrate probes (Veeco & Seiko Instruments) and the spring constant was 0.57 or 11 N/m. A glass bead (Park Science) of 20 μm diameter was attached to the tip of the cantilever using an epoxy resin (Vantico), and kept at room temperature for 24 h. The AFM cantilevers with glass bead attached were mounted into the AFM, and the glass bead was immersed in 10 μl of tyrosinase-treated protein solutions (1.44 mg/ml BSA, Cell-Tak™, MGFP-1 or MGFP-5) (MGFP-5 or MGFP-151) for 10 min. The glass bead was brought into contact with clean glass surface, and a force-distance curve was obtained.(Time required for one cycle of the force-distance curve measurement was 5 s. 삽입)

Fig. 21 shows the adhesion force of recombinant MGFP-151 and MGFP-5 proteins with modified tyrosines. Recombinant mussel adhesive proteins MGFP-5 and MGFP-151 have average values of 540 nN and 500 nN respectively indicating strong adhesion force, whereas that of Cell-Tak was 250 nN, and that of BSA was around 30 nN. This result shows that tyrosinase-treated recombinant mussel adhesion proteins have strong adhesion ability.

Example 14: Measurement of cell adhesion property

Drosophila S2 cells (Invitrogen) were used.

S2 cells were grown at 27 °C in M3 medium (Shields and Sang M3 insect medium; Sigma, St. Louis, MO) containing 10% IMS (insect medium supplement), 1% antibiotic-antimycotic (Invitrogen), and hygromycin 3 μl/ml. Tyrosinase-treated recombinant MGFP-5 protein, recombinant MGFP-151 protein, Cell-Tak, and BSA prepared from Example 10 were dropped onto sterilized slide glass (20 mm x 20 mm, Marienfeld, Germany) and incubated at 25°C for 30 minutes in a laminar flow hood and then washed two times with PBS. After washing, the coated slide glass was immersed in 100-mm cell culture dishes containing S2 cells at a concentration of 4×10^6 cells/ml showing 95% viability. After incubation at 27°C for 1 hr to 7 days, unattached cells were rinsed away with PBS, and cell viability and location of adhered protein was checked by trypan blue staining.

As a result, S2 cells were found to attach to regions where recombinant MGFP-5 and MGFP-151 proteins were coated, and the attached S2 cells survived for 7 days or more (Figs. 22 and 23).

Example 15: Examination of the chemical adhesive stability of MGFP-5 and MGFP-151

The recombinant proteins MGFP-5 and MGFP-151 were each spotted onto glass and Cell-Tak was spotted on as control. After drying, it was immersed in a solution consisting of 5 % acetic acid, 25 % methanol, and 70% water, and heated for

20 minutes at 85 °C. As a result, for the adhesive proteins coated on the surface of glass or acrylic plates, it was found that upon leaving at high temperatures under solvent conditions MGFP-5 became detached while MGFP-151 continued to stay attached.

Also, each was coated onto glass or acrylic plates and adhesive stability was measured through SEM.

Figs. 24A to C are photographs of the substrate surfaces coated with recombinant MGFP-5 and MGFP-151 proteins, where A is a 2500X enlargement, B is a 10000x enlargement, and C is a 35000X enlargement. On observation, the surface coated with MGFP-151 was found to be smooth, whereas the surface coated with MGFP-5 was found to be a little bit rough. This difference is thought to be a difference in the degree of cross-linking.

【Sequence list pretext】

SEQ ID NOs: 1 to 4 are primer sequences.

SEQ ID NO: 5 is the cDNA of MGFP-5 protein isolated from *Mytilus galloprovincialis.*

SEQ ID NO: 6 is the protein sequence of MGFP-5 protein isolated from *Mytilus galloprovincialis.*

SEQ ID NO: 7 is the nucleotide sequence of 6 tandem repeats of a partial sequence of MEFP-5 protein isolated from *Mytilus edulis.*

SEQ ID NO: 8 is the amino acid sequence of 6 tandem repeats of a partial sequence of MEFP-5 protein isolated from *Mytilus edulis.*

SEQ ID NO: 9 is the nucleotide sequence encoding the recombinant adhesive protein MGFP-15 constructed from FP-1 and MGFP-5.

SEQ ID NO: 10 is the amino acid sequence of the recombinant adhesive protein MGFP-15 constructed from FP-1 and MGFP-5.

SEQ ID NO: 11 is the nucleotide sequence encoding the recombinant adhesive protein MGFP-015 constructed from FP-1 and MGFP-5.

SEQ ID NO: 12 is the amino acid sequence of the recombinant adhesive protein MGFP-015 constructed from FP-1 and MGFP-5.

SEQ ID NO: 13 is the nucleotide sequence encoding the recombinant adhesive protein MGFP-151 constructed from FP-1 and MGFP-5.

SEQ ID NO: 14 is the amino acid sequence of the recombinant adhesive protein MGFP-151 constructed from FP-1 and MGFP-5.

SEQ ID NO: 15 is the nucleotide sequence of the construct inserted into the pMDG05 vector for the expression of MGFP-5.

SEQ ID NO: 16 is the amino acid sequence of the adhesive protein expressed from the construct inserted into the pMDG05 vector for the expression of MGFP-5.

SEQ ID NO: 17 is the nucleotide sequence of the construct inserted into the pMDG150 vector for the expression of MGFP-15.

SEQ ID NO: 18 is the amino acid sequence of the adhesive protein expressed from the construct inserted into the pMDG150 vector for the expression of MGFP-15.

SEQ ID NO: 19 is the nucleotide sequence of the construct inserted into the

pMDG051 vector for the expression of MGFP-51.

SEQ ID NO: 20 is the amino acid sequence of the adhesive protein expressed from the construct inserted into the pMDG051 vector for the expression of MGFP-51.

SEQ ID NO: 21 is the nucleotide sequence of the construct inserted into the pMDG151 vector for the expression of MGFP-151.

SEQ ID NO: 22 is the amino acid sequence of the adhesive protein expressed from the construct inserted into the pMDG151 vector for the expression of MGFP-151.

SEQ ID Nos: 23 to 24 are primer sequences.

SEQ ID NO: 25 is a partial sequence of FP-1.

SEQ ID Nos: 26 to 31 are nucleotide sequences encoding AKPSYPPTYK which is a partial sequence of FP-1.

SEQ ID NO: 32 is the primer sequence of the T3 promoter.

SEQ ID NO: 33 is the primer sequence of the T7 promoter.

SEQ ID Nos: 34 to 35 are primer sequences.

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO : CHUNG, Sung-Kee
Pohang University of Science & Technology,
#San 31, Hyoja-dong, Nam-gu, Pohang-si, Kyungbuk 790-784,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: *Escherichia coli* Top10/pMDG05	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCTC 10291BP

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:
[x] a scientific description
[ ] a proposed taxonomic designation
(Mark with a cross where applicable)

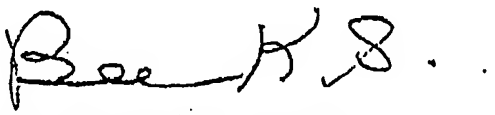
III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on **June 20 2002.**

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s):  BAE, Kyung Sook, Director Date: **June 25 2002**

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

Issued pursuant to Rule 7.1

TO : PARK, Chan-Mo
Pohang University of Science & Technology,
#San 31, Hyoja-dong, Nam-gu, Pohang-si, Kyungsangbuk-do 790-784,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Escherichia coli Top10/pENG151	KCTC 10766BP

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:
[x] a scientific description
[ ] a proposed taxonomic designation
(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on **Jan 19 2005.**

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s): PARK, Yong-Ha Director Date: Jan. 24 2005

WHAT IS CLAIMED IS:

1. An adhesive protein comprising an amino acid sequence shown in SEQ ID NO: 6.

2. The adhesive protein of Claim 1, wherein the adhesive protein further comprises a peptide for improving a physicochemical property of the adhesive protein which is attached to a carboxy- and/or amino-termini of the protein.

3. The adhesive protein of Claim 2, wherein the physicochemical property is selected from the group consisting of solubility, adhesion force, cross-linking, and improvement in protein expression, purification, and recovery rate.

4. The adhesive protein of Claim 2, wherein the peptide is derived from an adhesive protein.

5. The adhesive protein of Claim 4, wherein the adhesive protein is derived from a mussel adhesive protein.

6. The adhesive protein of Claim 2, wherein the peptide is an amino acid sequence as shown in SEQ ID NO: 25 tandemly repeated 1 to 10 times.

7. The adhesive protein of Claim 6, wherein the adhesive protein comprises an amino acid sequence selected from the group consisting of an amino acid sequence as shown in SEQ ID NO: 10, an amino acid sequence as shown in SEQ ID NO: 12, and an amino acid sequence as shown in SEQ ID NO: 14.

8. The adhesive protein of Claim 2, wherein the peptide comprises 6 histidine residues.

9. The adhesive protein of Claim 8, wherein the adhesive protein comprises an amino acid sequence selected from the group consisting of an amino acid

sequence as shown in SEQ ID NO: 16, an amino acid sequence as shown in SEQ ID NO: 18, an amino acid sequence as shown in SEQ ID NO: 20, and an amino acid sequence as shown in SEQ ID NO: 22.

10. A polynucleotide comprising a nucleotide sequence encoding an adhesive protein according to Claim 1.

11. The polynucleotide of Claim 10, wherein the nucleotide sequence encoding the adhesive protein comprises a nucleotide sequence as shown in SEQ ID NO: 5.

12. A polynucleotide comprising a nucelotide sequence encoding the adhesive protein according to Claim 2 wherein a peptide for improving a physicochemical property of the adhesive protein is attached to a carboxy- and/or amino-termini of the adhesive protein consisting of an amino acid sequence as shown in SEQ ID NO: 6.

13. The polynucleotide of Claim 12, wherein the peptide is an amino acid sequence as shown in SEQ ID NO: 25 tandemly repeated 1 to 10 times.

14. The polynucleotide of Claim 13, wherein a nucletide sequence encoding the peptide is selected from the group consisting of nucleotide sequences shown in SEQ ID Nos: 26 to 31 tandemly repeated 1 to 10 times.

15. The polynucleotide of Claim 13, wherein the polynucleotide is selected from the group consisting of a nucletide sequence as shown in SEQ ID NO: 9, a nucletide sequence as shown in SEQ ID NO: 11, and a nucletide sequence as shown in SEQ ID NO: 13.

16. A polynucleotide comprising a nucleotide sequence encoding the

adhesive protein according to Claim 8.

17. The polynucleotide of Claim 16, wherein the nucleotide sequence encoding the adhesive protein is selected from the group consisting of a nucletide sequence as shown in SEQ ID NO: 15, a nucletide sequence as shown in SEQ ID NO: 17, a nucletide sequence as shown in SEQ ID NO: 19, and a nucleotide sequence as shown in SEQ ID NO: 21.

18. A vector that comprises operably a nucletide sequence encoding an adhesive protein according to any one of Claims 1 to 9.

19. The vector of Claim 18, wherein the nucletide sequence encoding the adhesive protein is selected from the group consisting of a nucleotide sequence as shown in SEQ ID NO: 5, a nucleotide sequence as shown in SEQ ID NO: 9, a nucleotide sequence as shown in SEQ ID NO: 11, a nucleotide sequence as shown in SEQ ID NO: 13, a nucleotide sequence as shown in SEQ ID NO: 15, a nucleotide sequence as shown in SEQ ID NO: 17, a nucleotide sequence as shown in SEQ ID NO: 19, and a nucleotide sequence as shown in SEQ ID NO: 21.

20. The vector of Claim 18, wherein the vector is pMDG05 (KCTC 10291BP) or pENG151(KCTC 10766BP).

21. A transformant transformed with the vector according to Claim 18, wherein the transformant is selected from the group consisting of prokaryotes, eukaryotes, and eukaryote-derived cells.

22. The transformant of Claim 21, wherein the prokaryote is *E. coli* or *Bacillus sp.*

23. The transformant of Claim 21, wherein the eukaryote is selected from

the group consisting of yeast, insects, plants, and animals.

24. The transformant of Claim 21, wherein the eukaryote-derived cells are selected from the group consisting of plant cells, insect cells, and mammalian cells.

25. A method of producing an adhesive protein comprising the steps of:

(a) constructing a vector that comprises operably a nucleotide encoding the adhesive protein according to Claim 1;

(b) constructing a transformant by transforming the vector into a host cell; and

(c) producing recombinant adhesive protein by culturing the transformant.

26. The method of Claim 25, wherein the adhesive protein further comprises a peptide for improving a physicochemical property of the adhesive protein attached to a carboxy- and/or amino-termini of the protein.

27. The method of Claim 26, wherein the physicochemical property is selected from the group consisting of solubility, adhesion force, cross-linking, and improvement in protein expression, purification, and recovery rate.

28. The method of Claim 26, wherein the peptide is derived from an adhesive protein.

29. The method of Claim 28, wherein the adhesive protein is derived from a mussel adhesive protein.

30. The method of Claim 26, wherein the peptide is an amino acid sequence as shown in SEQ ID NO: 25 tandemly repeated 1 to 10 times.

31. The method of Claim 30, wherein the adhesive protein comprises an amino acid sequence selected from the group consisting of an amino acid sequence

as shown in SEQ ID NO: 10, an amino acid sequence as shown in SEQ ID NO: 12, and an amino acid sequence as shown in SEQ ID NO: 14.

32. The method of Claim 26, wherein the peptide consists of 6 histidine residues.

33. The method of Claim 32, wherein the adhesive protein comprises an amino acid sequence selected from the group consisting of an amino acid sequence as shown in SEQ ID NO: 16, an amino acid sequence as shown in SEQ ID NO: 18, an amino acid sequence as shown in SEQ ID NO: 20, and an amino acid sequence as shown in SEQ ID NO: 22.

34. A method of purifying adhesive protein comprising the steps of:

(a) lysing the transformant according to Claim 21, and then centrifuging it to isolate each of the supernatant and pellet;

(b) making a suspension by adding acidic organic solvent to the pellet and suspending it; and

(c) centrifuging the suspension to isolate the supernatant.

35. The method of Claim 34, wherein the acidic organic solvent has a pH of 3 to 6.

36. The method of Claim 34, wherein the acidic organic solvent is one or more selected from the group consisting of acetic acid, citric acid, and lactic acid.

37. The method of Claim 36, wherein the acetic acid is a 5 to 30 (v/v) % aqueous solution.

38. The method of Claim 34, wherein gel filtration chromatography is further carried out on the supernatant of step (c).

39. An adhesive comprising an adhesive protein according to any one of Claims 1 to 9 as an active component.

40. The adhesive of Claim 39, wherein 5 % to 100 % of the total number of tyrosine residues in the adhesive protein is modified to 3,4-dihydroxyphenyl-L-alanine (DOPA)

41. The adhesive of Claim 39, wherein the adhesive adheres to a substrate selected from the group consisting of plastic, glass, metal, eukaryotic cells, prokaryotic cells, and plant cell walls and lipids.

42. The adhesive of Claim 39, wherein the adhesive is applied to biological sample.

43. The adhesive of Claim 39, wherein the adhesive further comprises one or more material selected from the group consisting of surfactant, oxidant, and filler.

44. The adhesive of Claim 43, wherein the surfactant is sodium dodecylsulfate or sodium dodecylbenzensulfonate.

45. The adhesive of Claim 43, wherein the oxidant is tyrosinase or H_2O_2.

46. The adhesive of Claim 43, wherein the filler is selected from the group consisting of collagen, hyaluronic acid, condroitan sulfate, elastine, laminin, caseine, hydroxyapatite, and albumin, fibronectin, and hybrin.

47. The adhesive of Claim 39, wherein the adhesive is applied to substrates used in an underwater environment.

48. A method of adjusting the adhesion force of the adhesive according to Claim 39, wherein the method comprises a step of treating with a substance selected from the group consisting of oxidant, filler, and surfactant, or a step of controlling

the concentration of the adhesive protein which is an active component of the adhesive.

49. A coating agent containing an adhesive protein according to any one of Claims 1 to 9 as an active component.

FIG.1

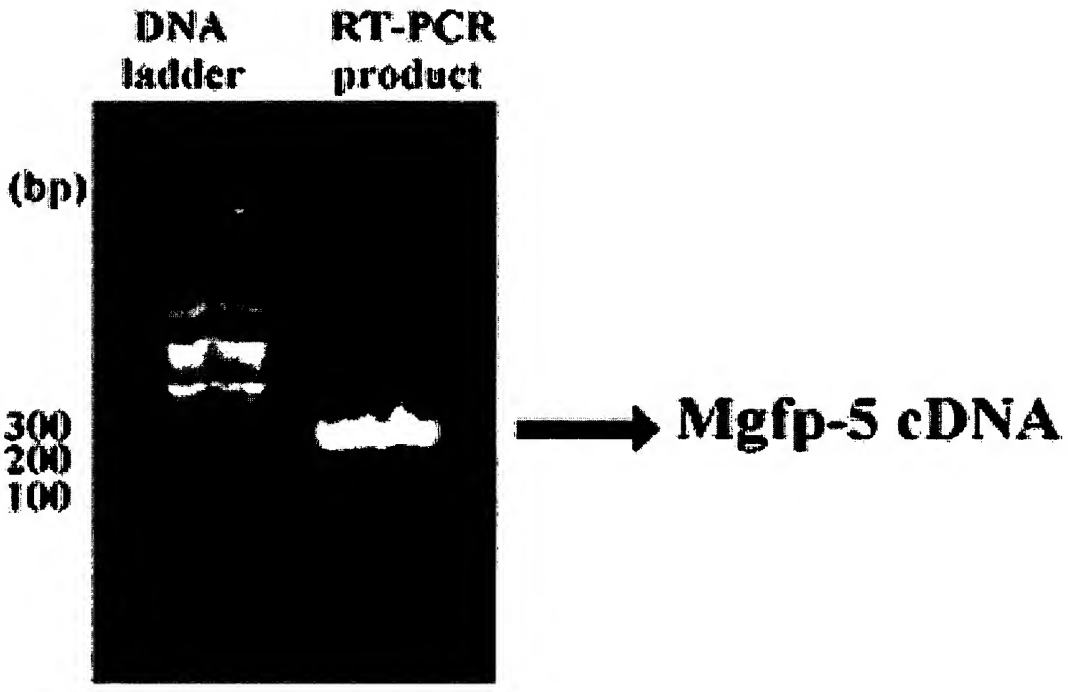


FIG.2

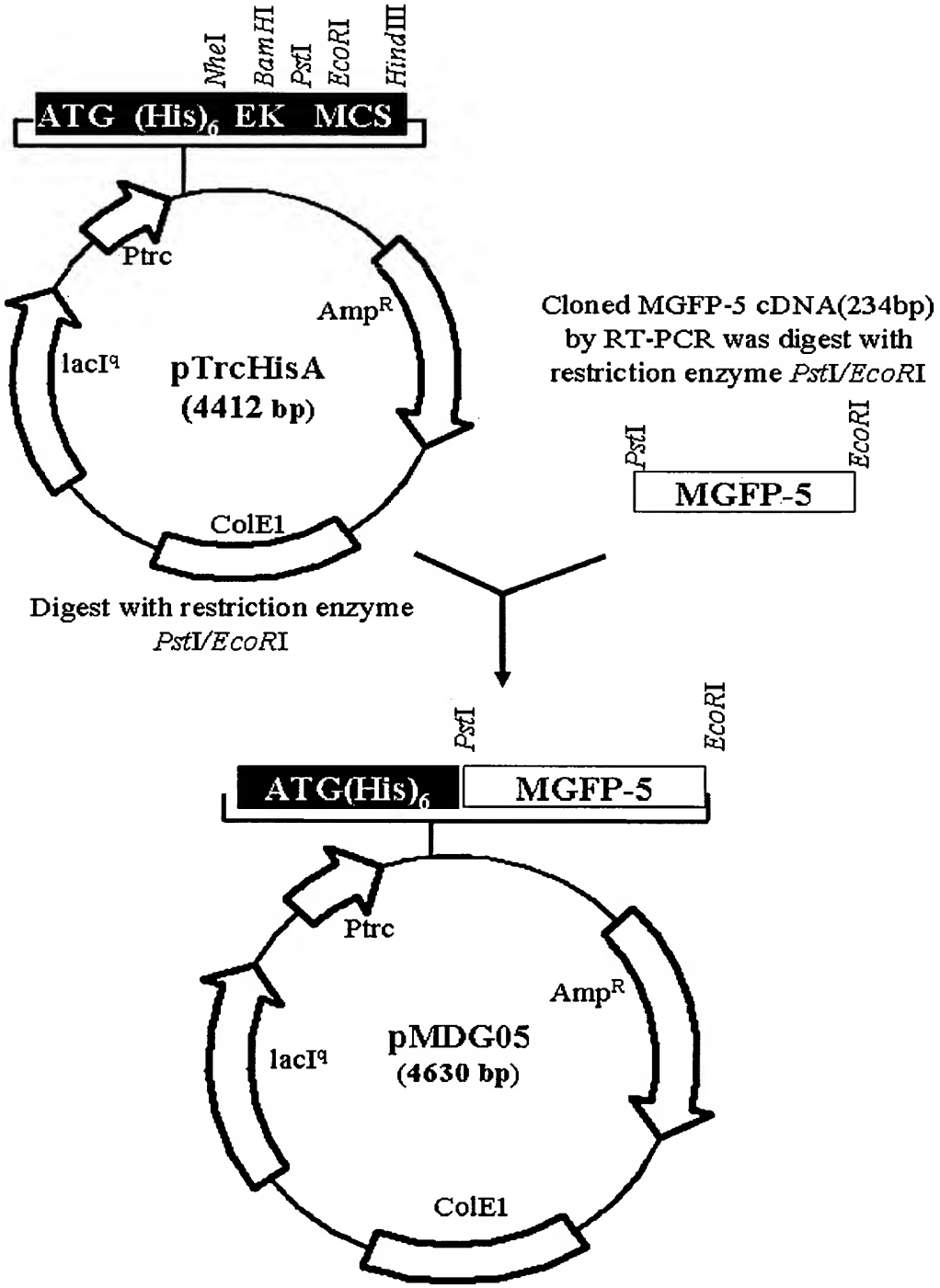

NheI
BamHI
PstI
EcoRI
HindIII
ATG (His)6 EK MCS
Ptrc
AmpR
lacIq
pTrcHisA
(4412 bp)
ColE1
Digest with restriction enzyme
PstI/EcoRI
Cloned MGFP-5 cDNA(234bp)
by RT-PCR was digest with
restriction enzyme PstI/EcoRI
PstI
EcoRI
MGFP-5
PstI
EcoRI
ATG(His)6
MGFP-5
Ptrc
AmpR
lacIq
pMDG05
(4630 bp)
ColE1

FIG.3

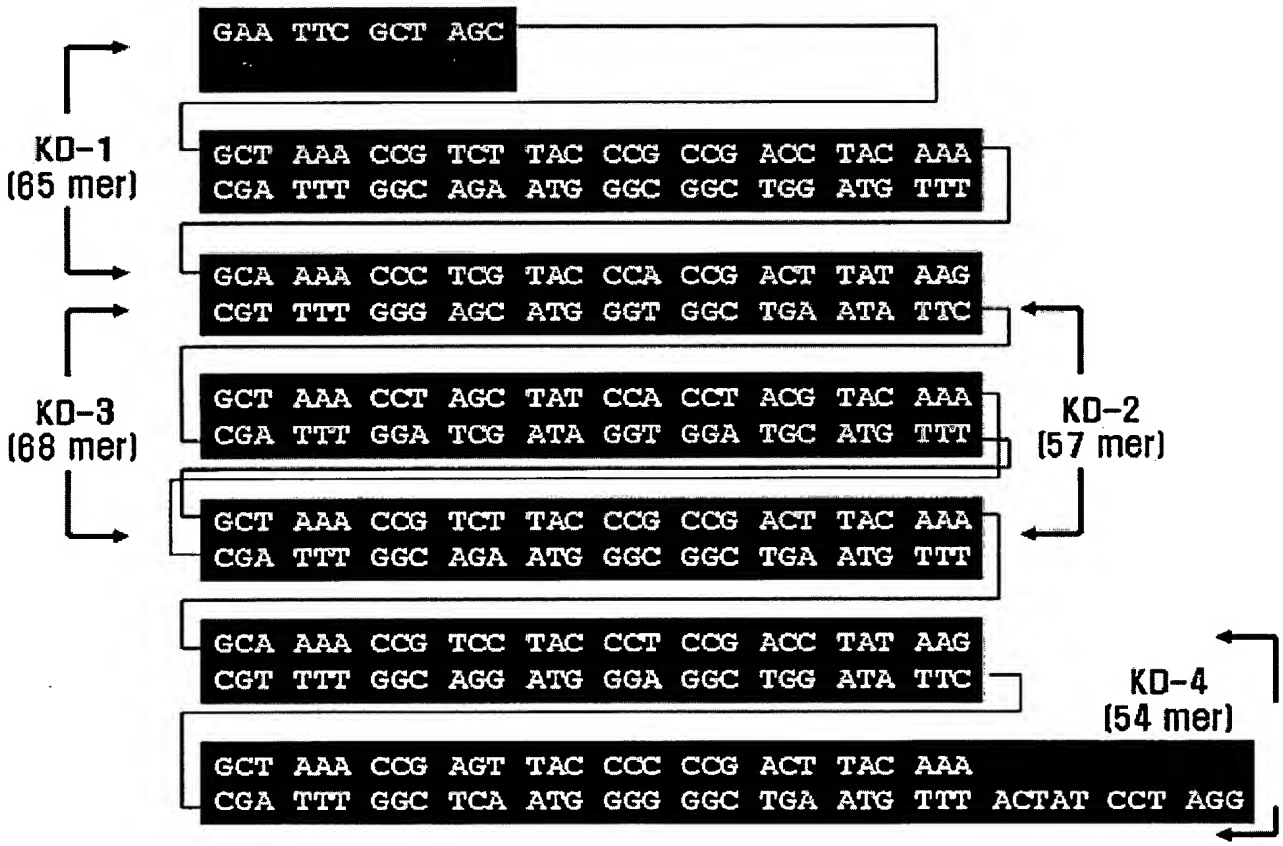

GAA TTC GCT AGC
KD-1
(65 mer)
GCT AAA CCG TCT TAC CCG CCG ACC TAC AAA
CGA TTT GGC AGA ATG GGC GGC TGG ATG TTT
GCA AAA CCC TCG TAC CCA CCG ACT TAT AAG
CGT TTT GGG AGC ATG GGT GGC TGA ATA TTC
KD-3
(68 mer)
GCT AAA CCT AGC TAT CCA CCT ACG TAC AAA
CGA TTT GGA TCG ATA GGT GGA TGC ATG TTT
KD-2
(57 mer)
GCT AAA CCG TCT TAC CCG CCG ACT TAC AAA
CGA TTT GGC AGA ATG GGC GGC TGA ATG TTT
GCA AAA CCG TCC TAC CCT CCG ACC TAT AAG
CGT TTT GGC AGG ATG GGA GGC TGG ATA TTC
KD-4
(54 mer)
GCT AAA CCG AGT TAC CCC CCG ACT TAC AAA
CGA TTT GGC TCA ATG GGG GGC TGA ATG TTT ACTAT CCT AGG

FIG.4

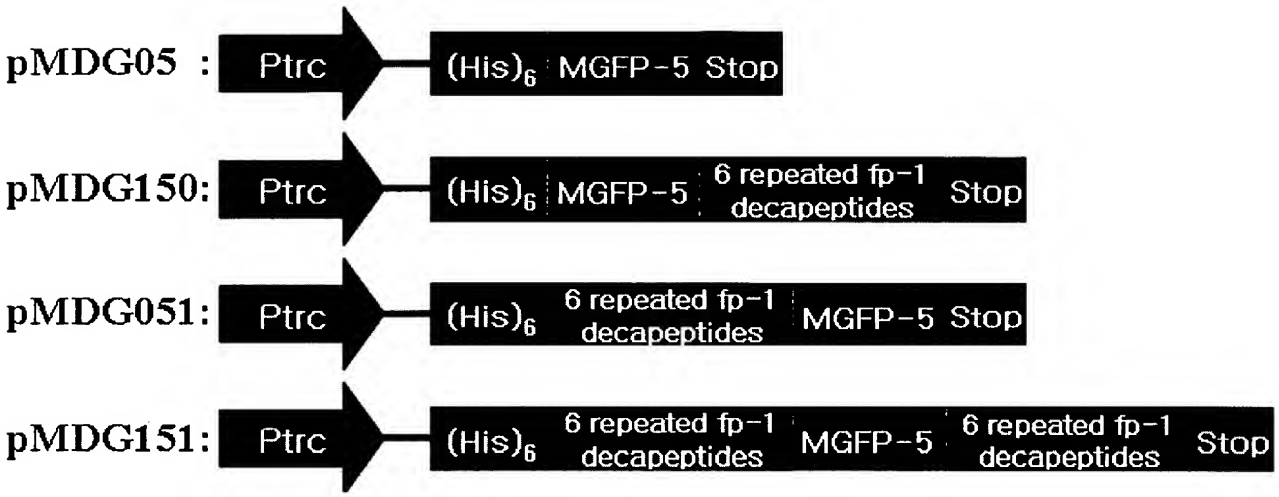

pMDG05 :
Ptrc
(His)6
MGFP-5
Stop
pMDG150:
Ptrc
(His)6
MGFP-5
6 repeated fp-1 decapeptides
Stop
pMDG051:
Ptrc
(His)6
6 repeated fp-1 decapeptides
MGFP-5
Stop
pMDG151:
Ptrc
(His)6
6 repeated fp-1 decapeptides
MGFP-5
6 repeated fp-1 decapeptides
Stop

FIG.5

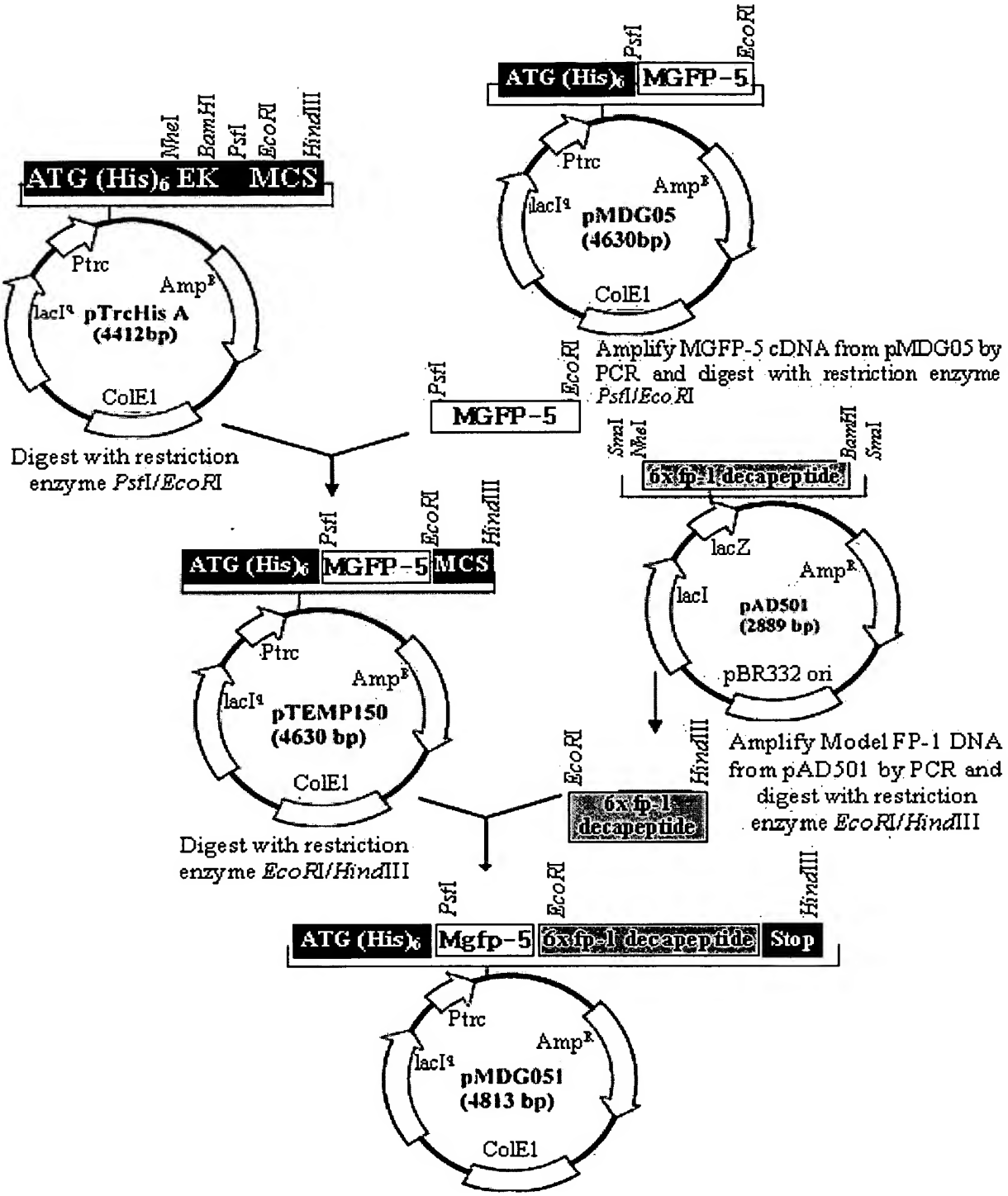

ATG (His)6 MGFP-5
PstI
EcoRI
Ptrc
Amp^R
lacI^q
pMDG05
(4630bp)
ColE1
Amplify MGFP-5 cDNA from pMDG05 by PCR and digest with restriction enzyme PstI/EcoRI
MluI
BamHI
PstI
EcoRI
HindIII
ATG (His)6 EK MCS
pTrcHis A
(4412bp)
Digest with restriction enzyme PstI/EcoRI
MGFP-5
SmaI
MluI
6xfp-1decapeptide
BamHI
SmaI
lacZ
lacI
pAD501
(2889 bp)
pBR332 ori
ATG (His)6
MGFP-5
MCS
pTEMP150
(4630 bp)
Amplify Model FP-1 DNA from pAD501 by PCR and digest with restriction enzyme EcoRI/HindIII
6x fp-1 decapeptide
Digest with restriction enzyme EcoRI/HindIII
ATG (His)6
Mgfp-5
6xfp-1 decapeptide
Stop
pMDG051
(4813 bp)

FIG.6

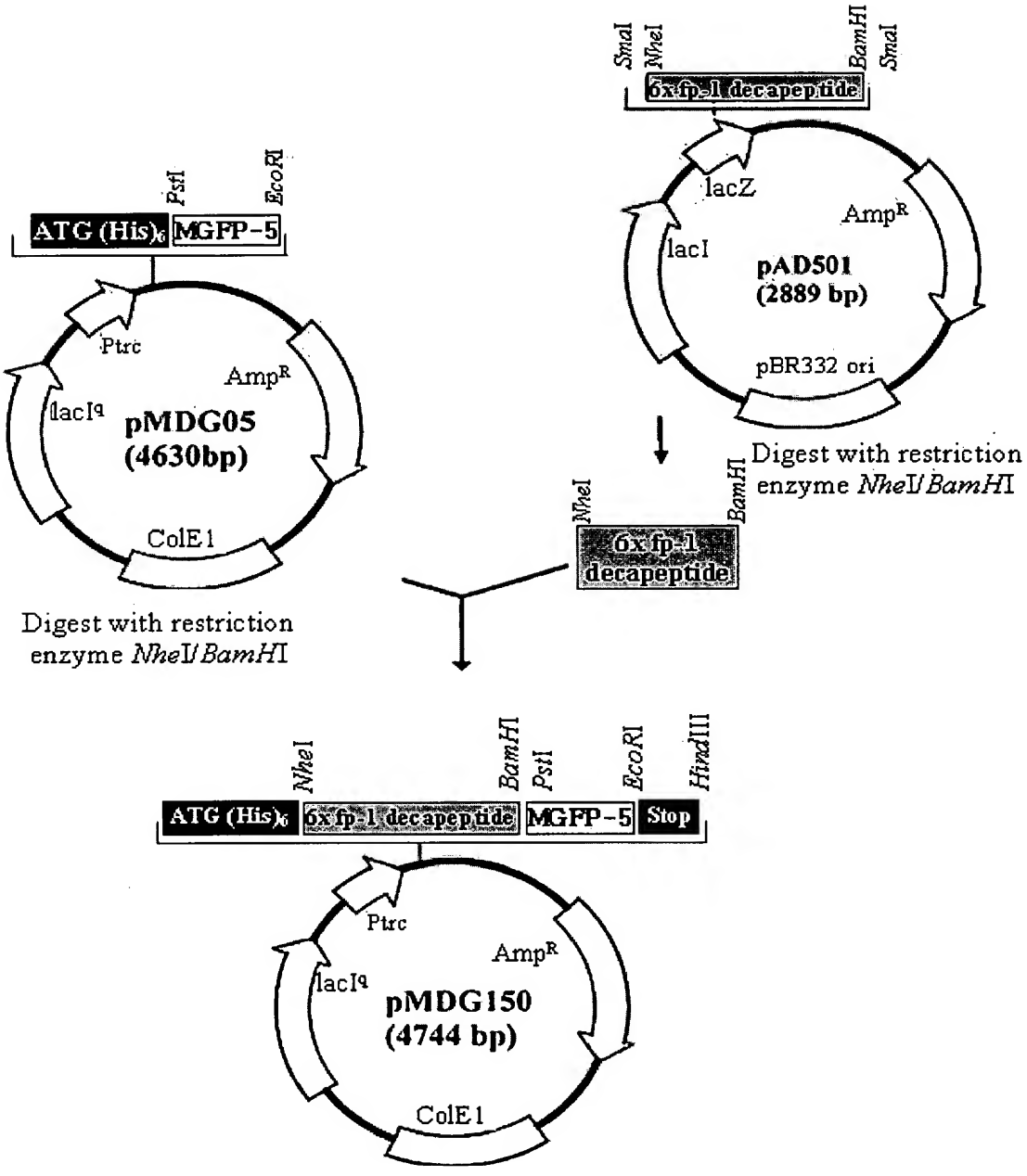
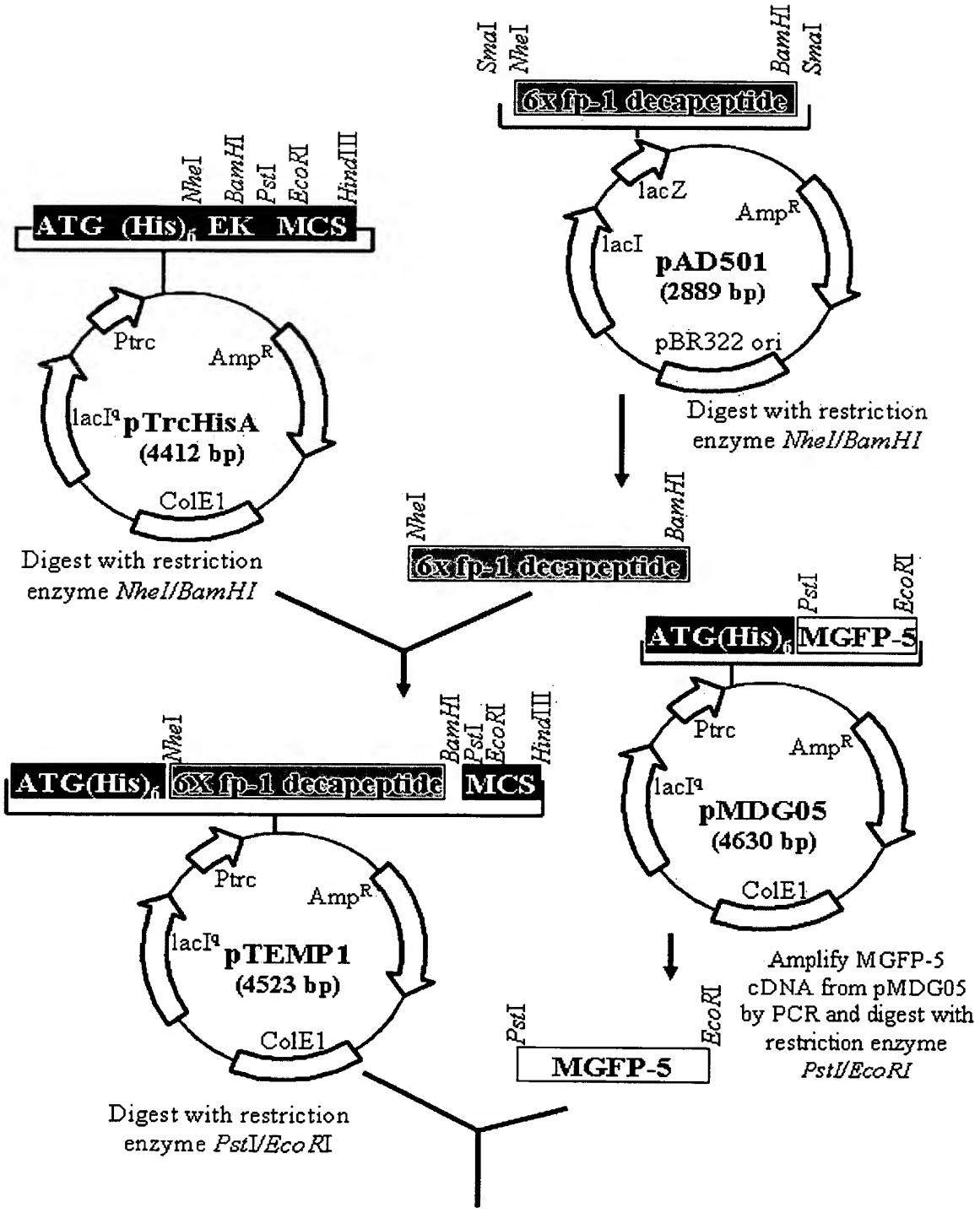

SmaI
NheI
BamHI
SmaI
6x fp-1 decapeptide
lacZ
Amp^R
lacI
pAD501
(2889 bp)
pBR332 ori
Digest with restriction enzyme NheI/BamHI
PstI
EcoRI
ATG (His)6
MGFP-5
Ptrc
Amp^R
lacI^q
pMDG05
(4630bp)
ColE1
Digest with restriction enzyme NheI/BamHI
NheI
BamHI
6x fp-1 decapeptide
NheI
BamHI
PstI
EcoRI
HindIII
ATG (His)6
6x fp-1 decapeptide
MGFP-5
Stop
Ptrc
Amp^R
lacI^q
pMDG150
(4744 bp)
ColE1

FIG.7


SmaI
NheI
BamHI
SmaI
6x fp-1 decapeptide
lacZ
Amp^R
lacI
pAD501
(2889 bp)
pBR322 ori
Digest with restriction enzyme NheI/BamHI
NheI
BamHI
PstI
EcoRI
HindIII
ATG (His)6 EK MCS
Ptrc
Amp^R
lacI^q pTrcHisA
(4412 bp)
ColE1
Digest with restriction enzyme NheI/BamHI
6x fp-1 decapeptide
PstI
EcoRI
ATG(His)6 MGFP-5
Ptrc
Amp^R
lacI^q
pMDG05
(4630 bp)
ColE1
NheI
BamHI
PstI
EcoRI
HindIII
ATG(His)6
6X fp-1 decapeptide
MCS
Ptrc
Amp^R
lacI^q pTEMP1
(4523 bp)
ColE1
Digest with restriction enzyme PstI/EcoRI
Amplify MGFP-5 cDNA from pMDG05 by PCR and digest with restriction enzyme PstI/EcoRI
PstI
EcoRI
MGFP-5

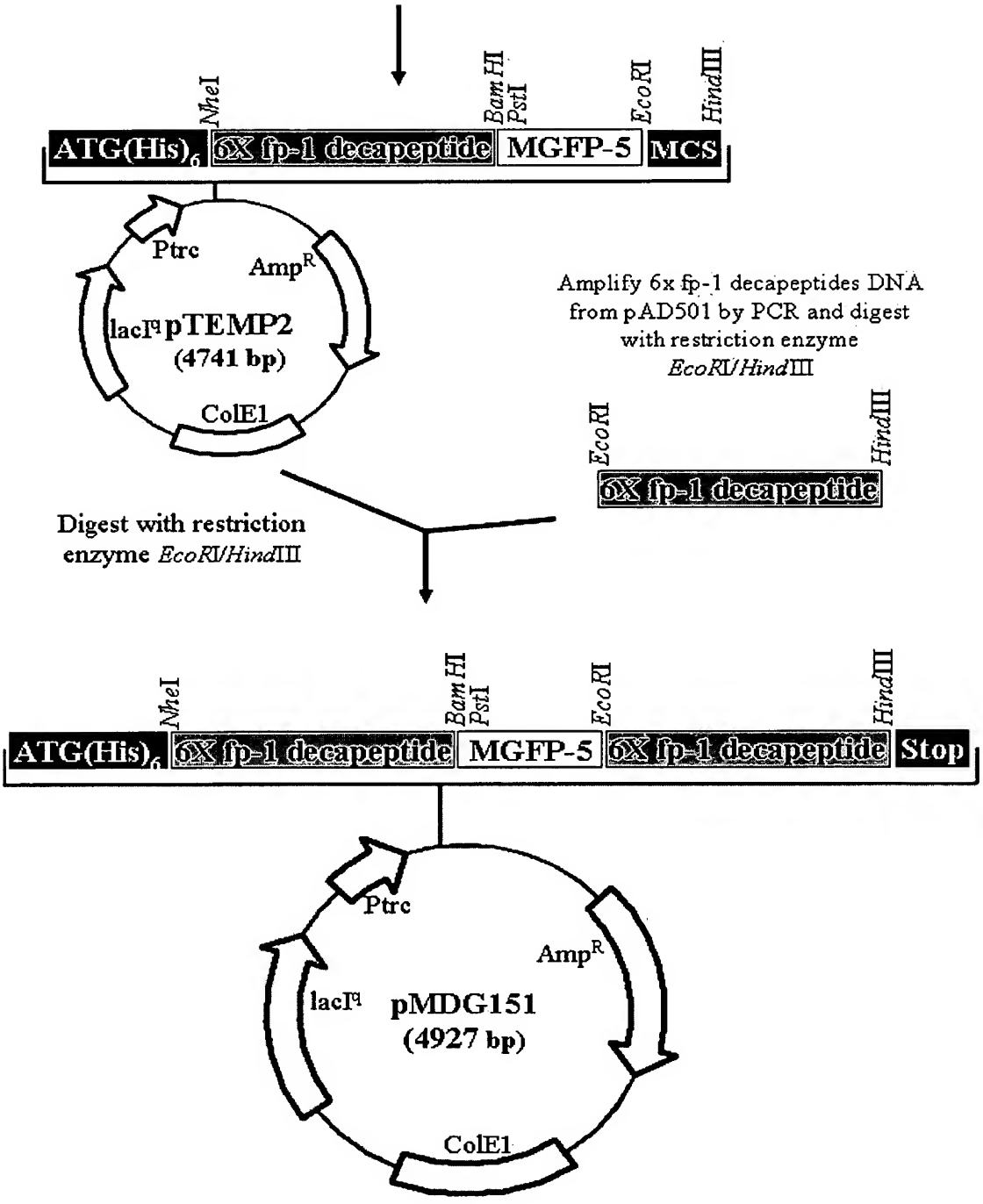

NheI
BamHI
PstI
EcoRI
HindIII
ATG(His)6
6X fp-1 decapeptide
MGFP-5
MCS
Ptrc
AmpR
lacIq pTEMP2
(4741 bp)
ColE1
Amplify 6x fp-1 decapeptides DNA from pAD501 by PCR and digest with restriction enzyme EcoRI/HindIII
EcoRI
HindIII
6X fp-1 decapeptide
Digest with restriction enzyme EcoRI/HindIII
NheI
BamHI
PstI
EcoRI
HindIII
ATG(His)6
6X fp-1 decapeptide
MGFP-5
6X fp-1 decapeptide
Stop
Ptrc
AmpR
lacIq
pMDG151
(4927 bp)
ColE1

FIG.8

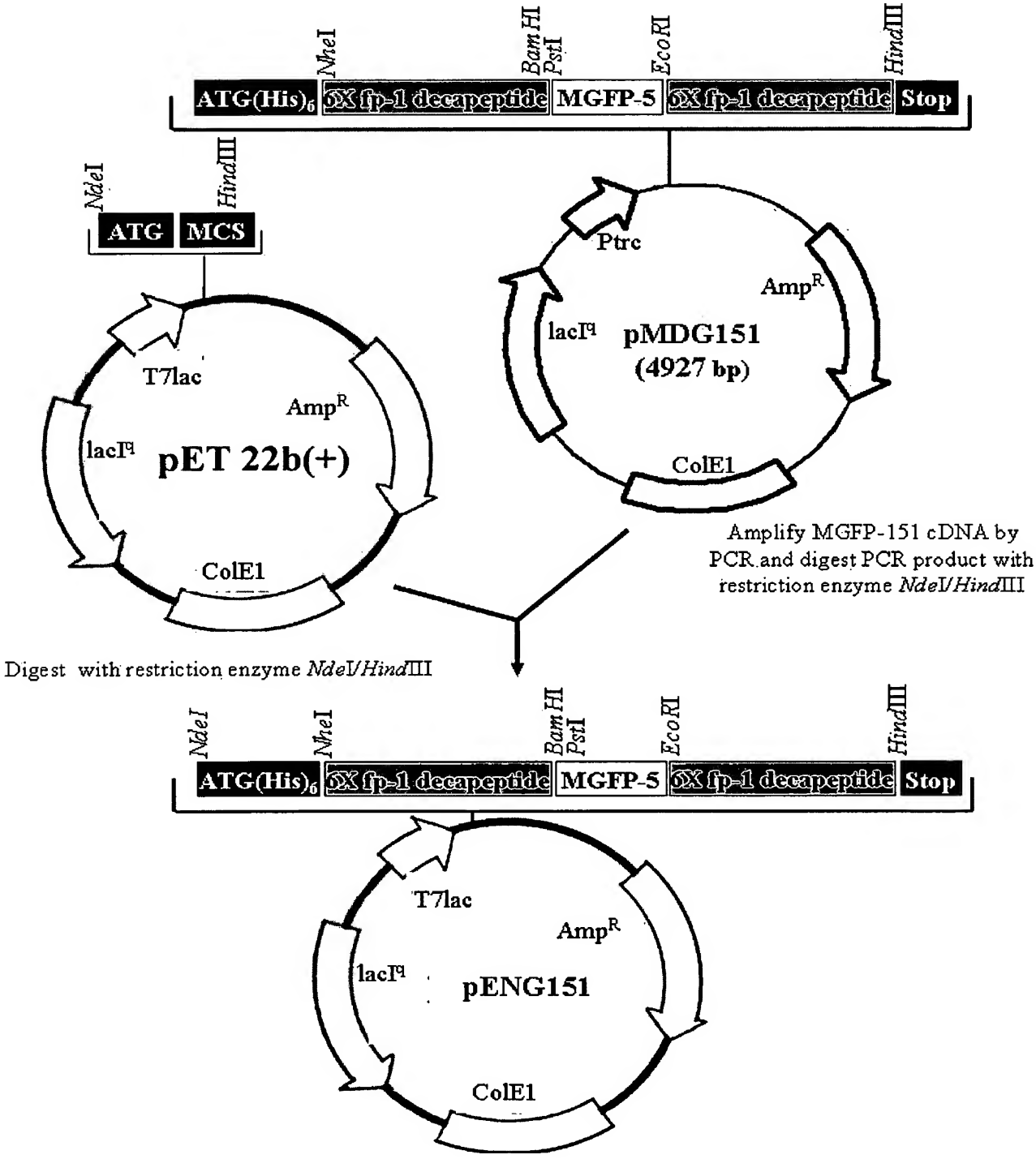

NheI
BamHI
PstI
EcoRI
HindIII
ATG(His)6
δX fp-1 decapeptide
MGFP-5
δX fp-1 decapeptide
Stop
NdeI
HindIII
ATG
MCS
Ptrc
AmpR
lacIq
pMDG151
(4927 bp)
ColE1
T7lac
AmpR
lacIq
pET 22b(+)
ColE1
Amplify MGFP-151 cDNA by PCR and digest PCR product with restriction enzyme NdeI/HindIII
Digest with restriction enzyme NdeI/HindIII
NdeI
NheI
BamHI
PstI
EcoRI
HindIII
ATG(His)6
δX fp-1 decapeptide
MGFP-5
δX fp-1 decapeptide
Stop
T7lac
AmpR
lacIq
pENG151
ColE1

FIG.9

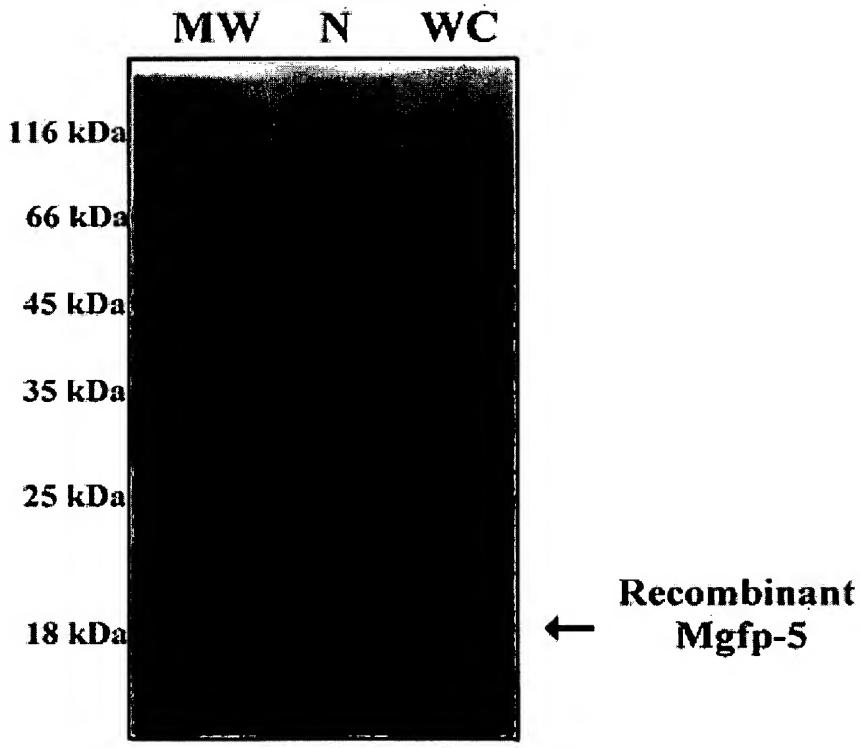

MW
N
WC
116 kDa
66 kDa
45 kDa
35 kDa
25 kDa
18 kDa
Recombinant
Mgfp-5

FIG. 10

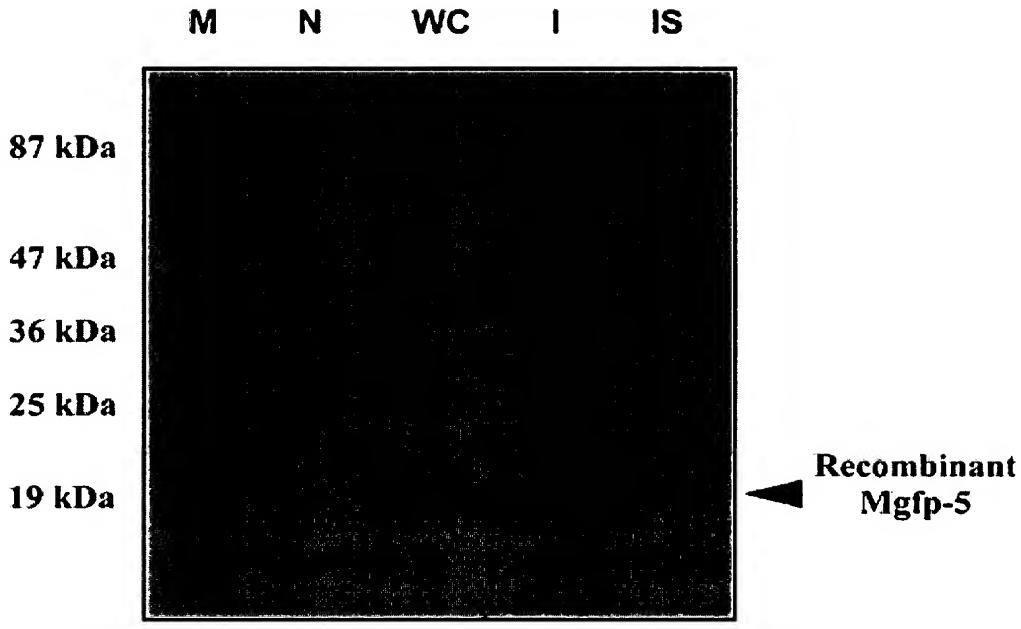


FIG.11

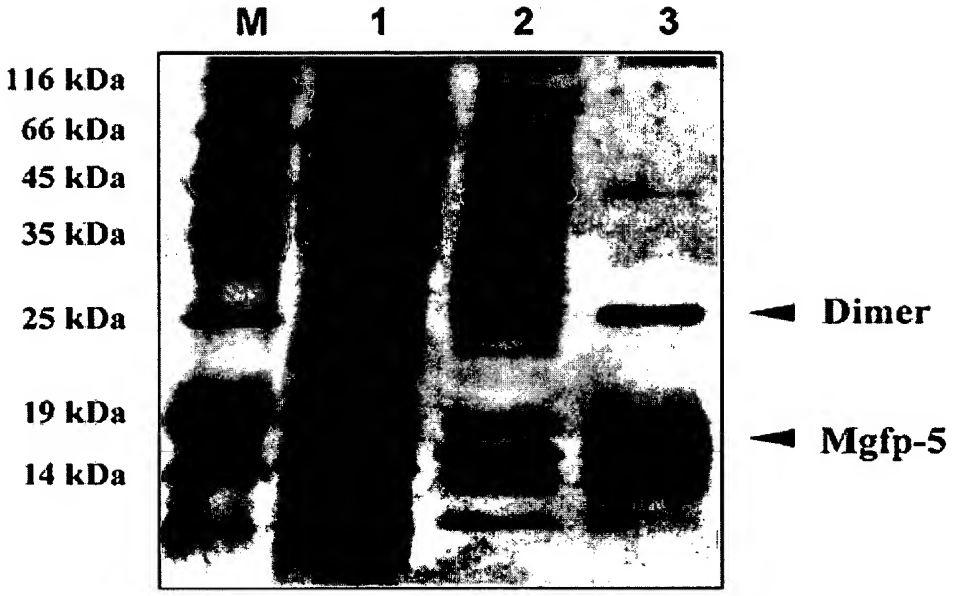

M
1
2
3
116 kDa
66 kDa
45 kDa
35 kDa
25 kDa
19 kDa
14 kDa
Dimer
Mgfp-5

FIG. 12

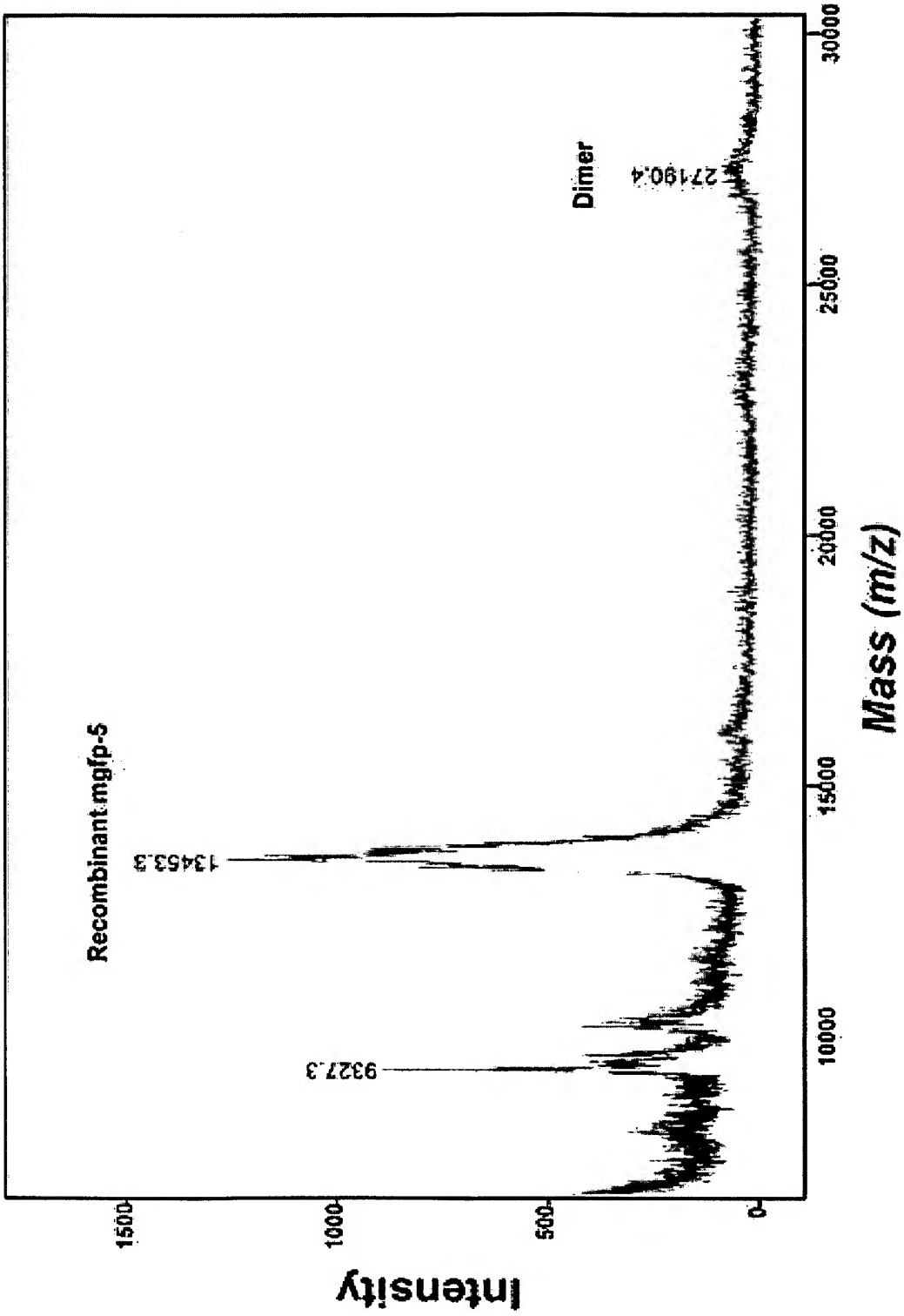

Recombinant mgfp-5
13453.3
9327.3
Dimer
27190.4
Intensity
1500
1000
500
0
10000
15000
20000
25000
30000
Mass (m/z)

FIG.13

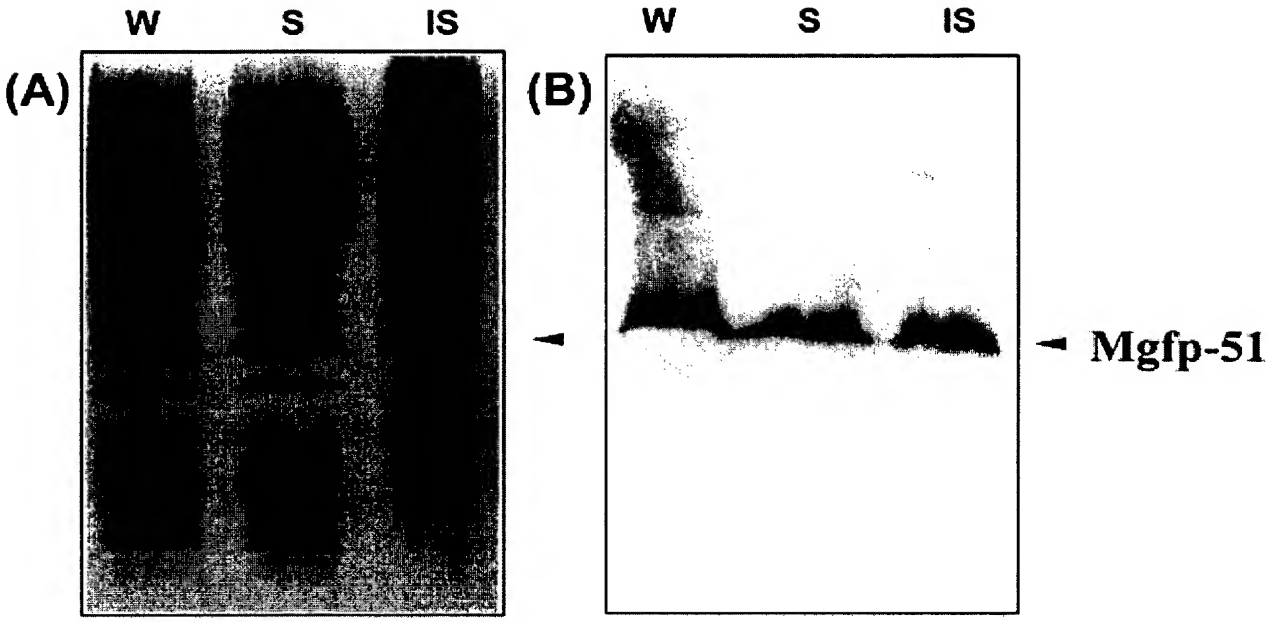


FIG. 14

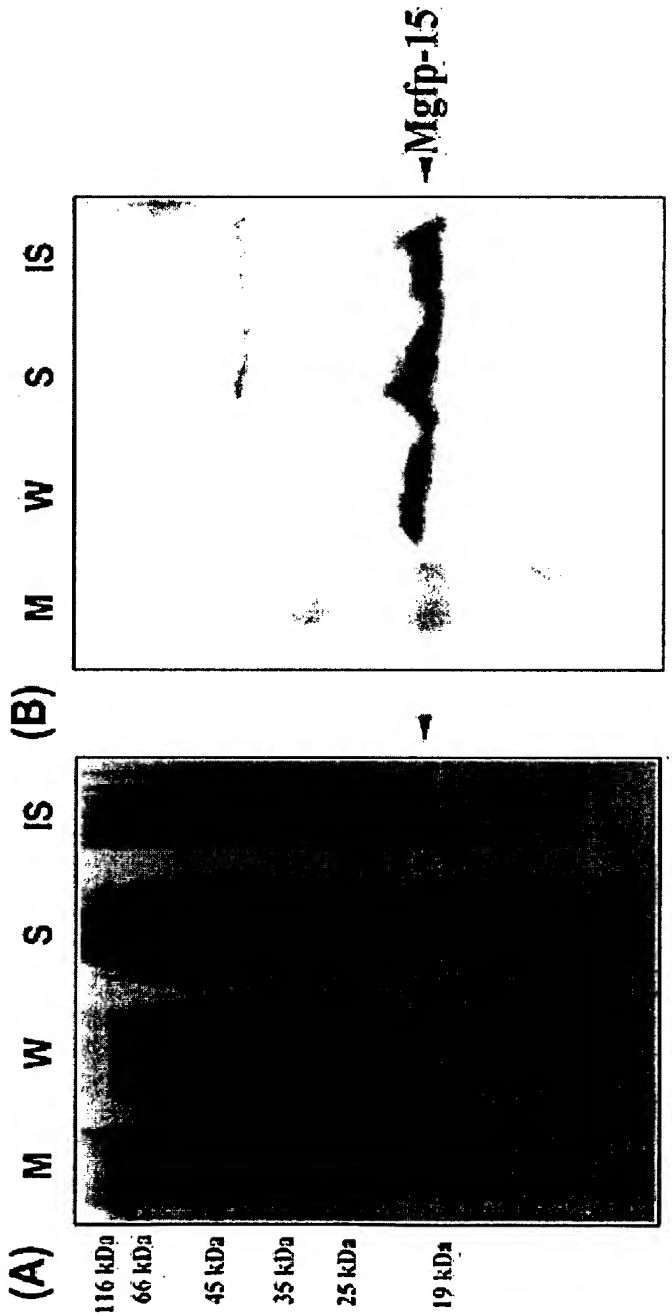


FIG.15

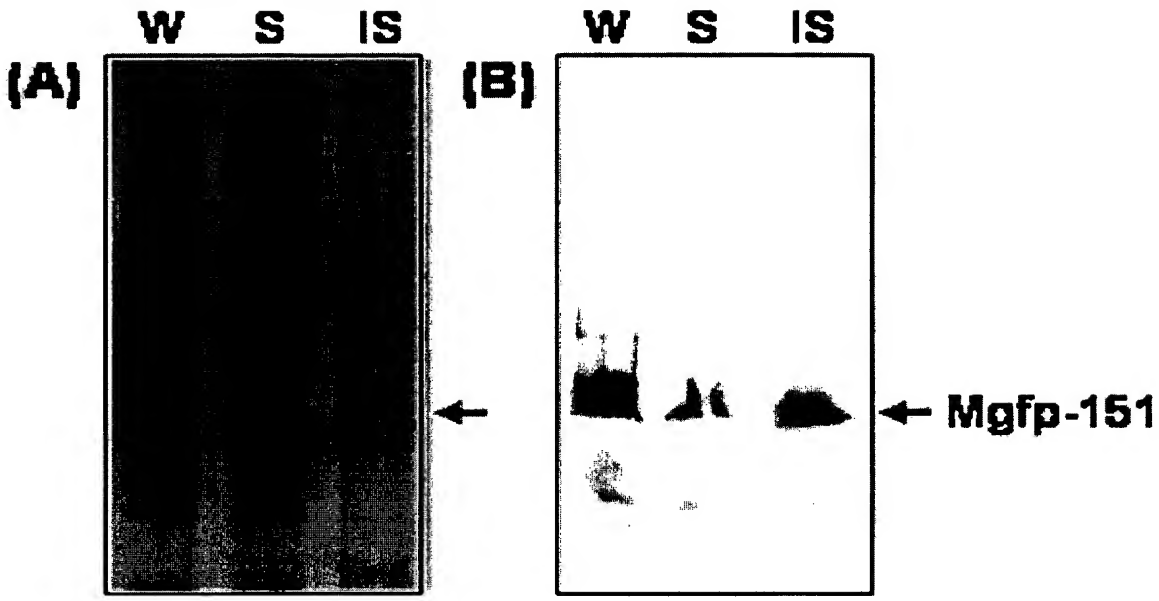


FIG. 16

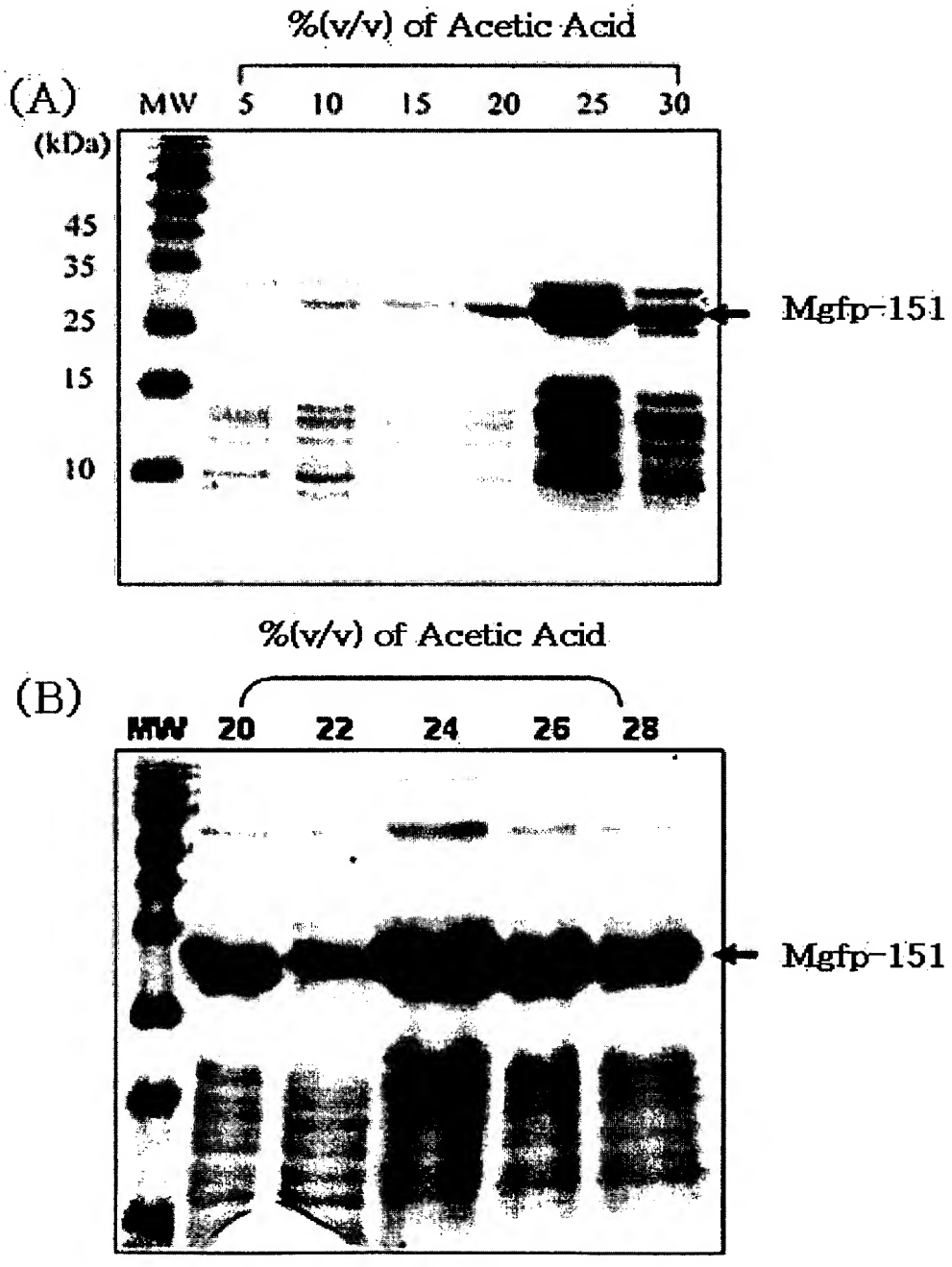

%(v/v) of Acetic Acid
(A)
MW
5
10
15
20
25
30
(kDa)
45
35
25
15
10
Mgfp-151
%(v/v) of Acetic Acid
(B)
MW
20
22
24
26
28
Mgfp-151

FIG. 17

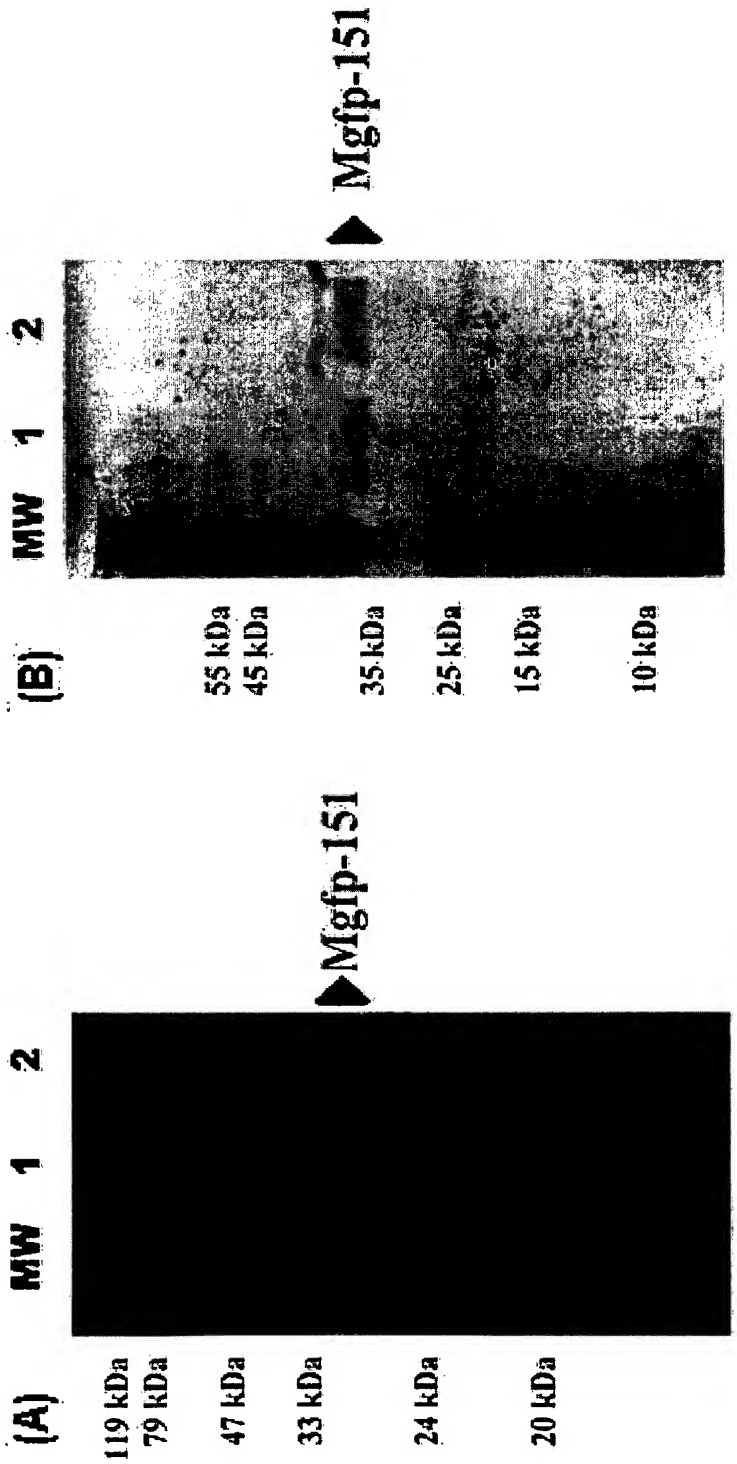

(A)
MW
1
2
Mgfp-151
119 kDa
79 kDa
47 kDa
33 kDa
24 kDa
20 kDa
(B)
MW
1
2
Mgfp-151
55 kDa
45 kDa
35 kDa
25 kDa
15 kDa
10 kDa

FIG. 18

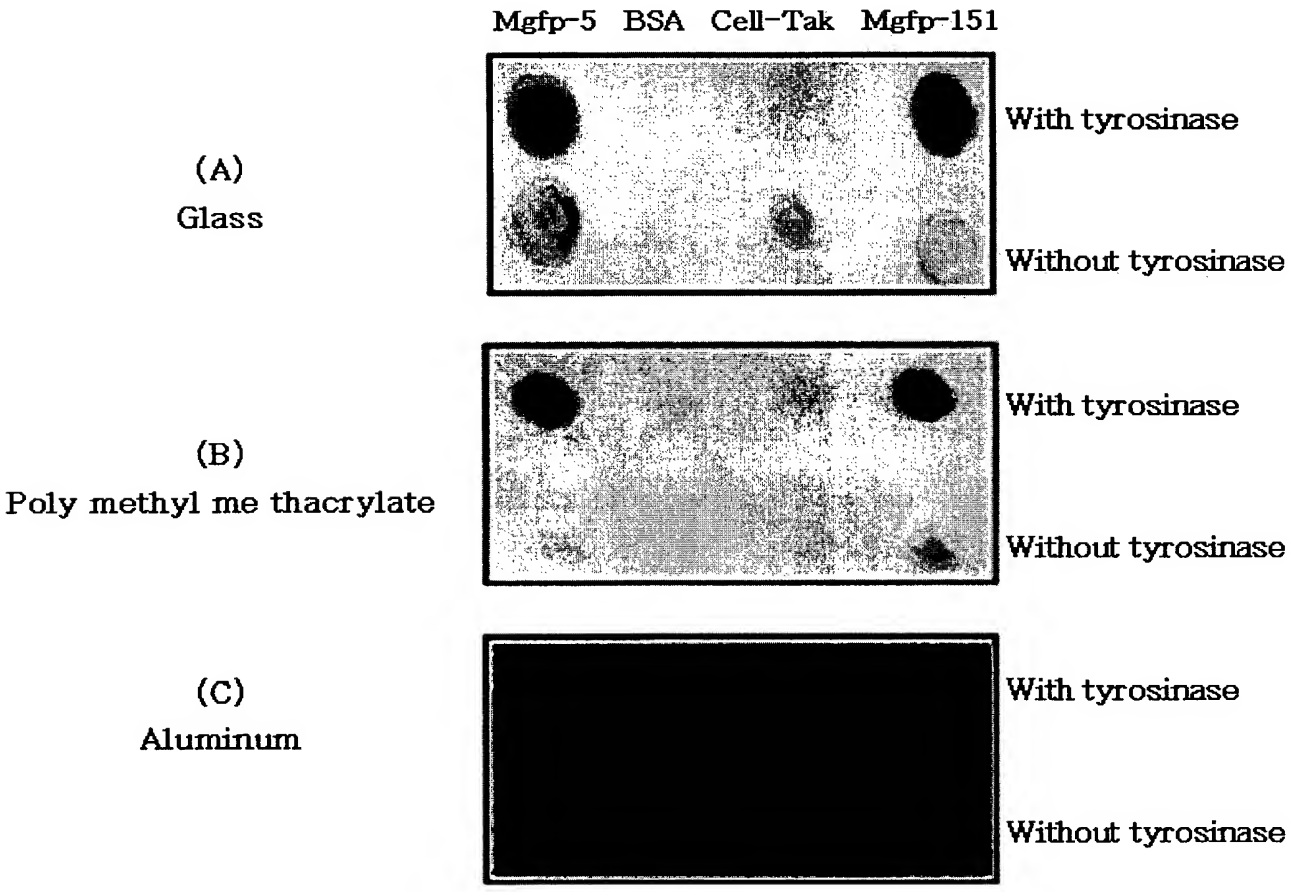

Mgfp-5 BSA Cell-Tak Mgfp-151
(A)
Glass
With tyrosinase
Without tyrosinase
(B)
Poly methyl me thacrylate
With tyrosinase
Without tyrosinase
(C)
Aluminum
With tyrosinase
Without tyrosinase

FIG. 19

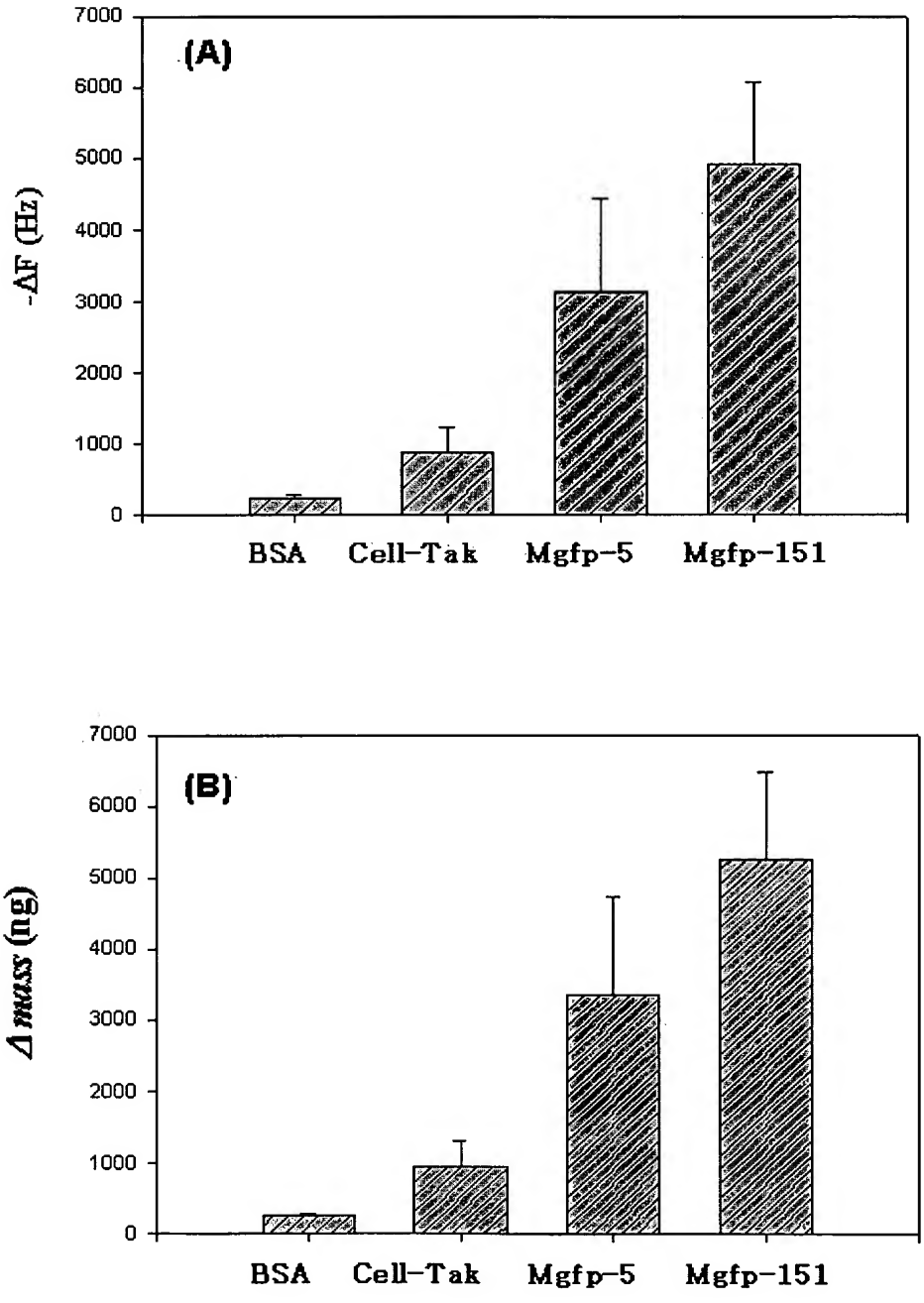

(A)
-ΔF (Hz)
7000
6000
5000
4000
3000
2000
1000
0
BSA
Cell-Tak
Mgfp-5
Mgfp-151
(B)
Δ mass (ng)
7000
6000
5000
4000
3000
2000
1000
0
BSA
Cell-Tak
Mgfp-5
Mgfp-151

FIG.20

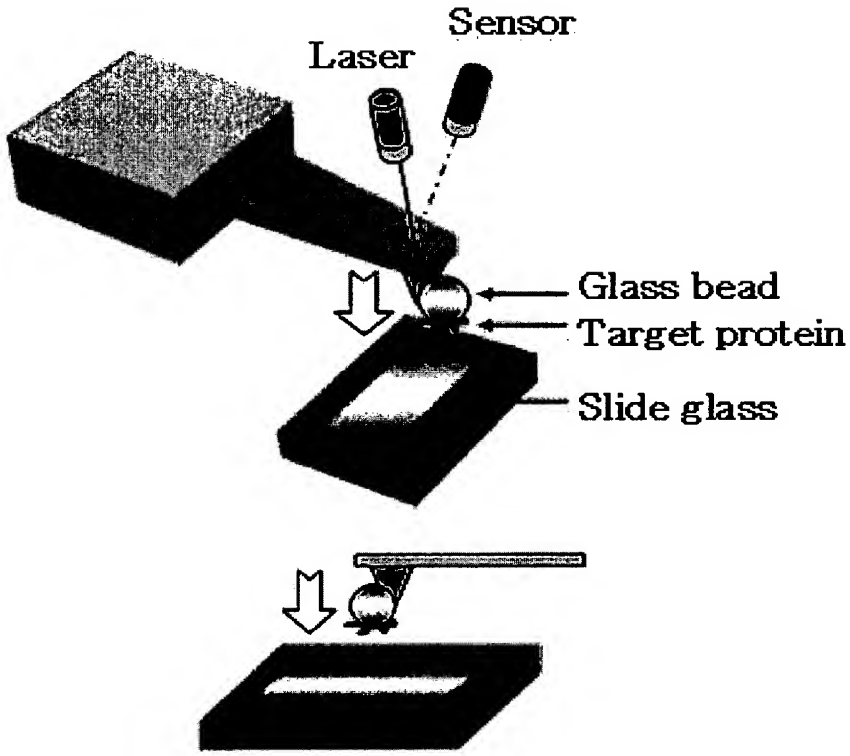


FIG.21

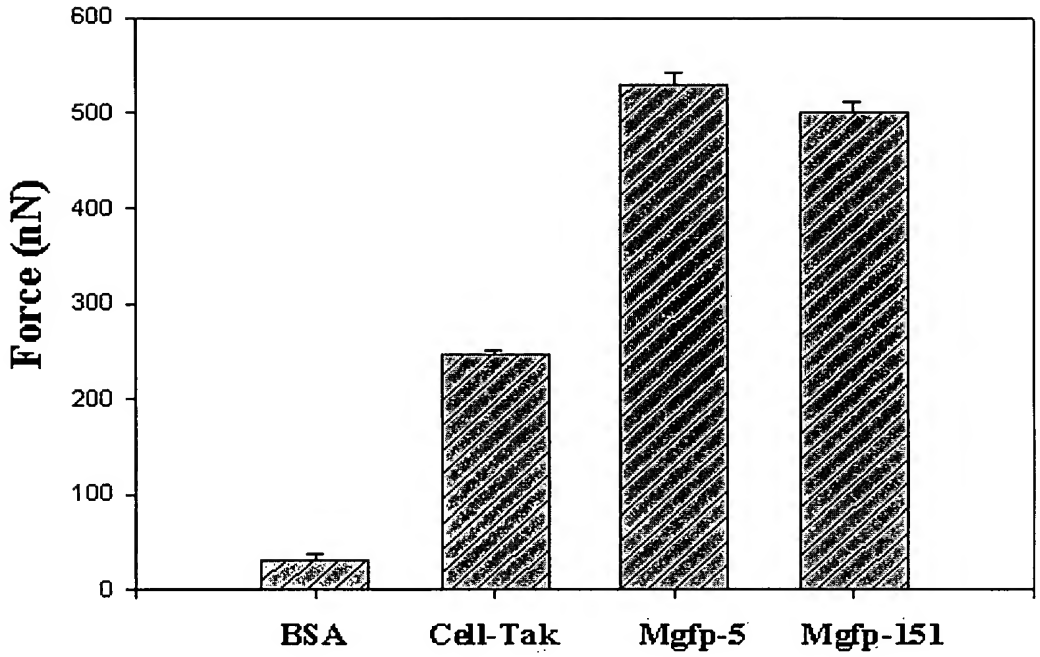


FIG.22

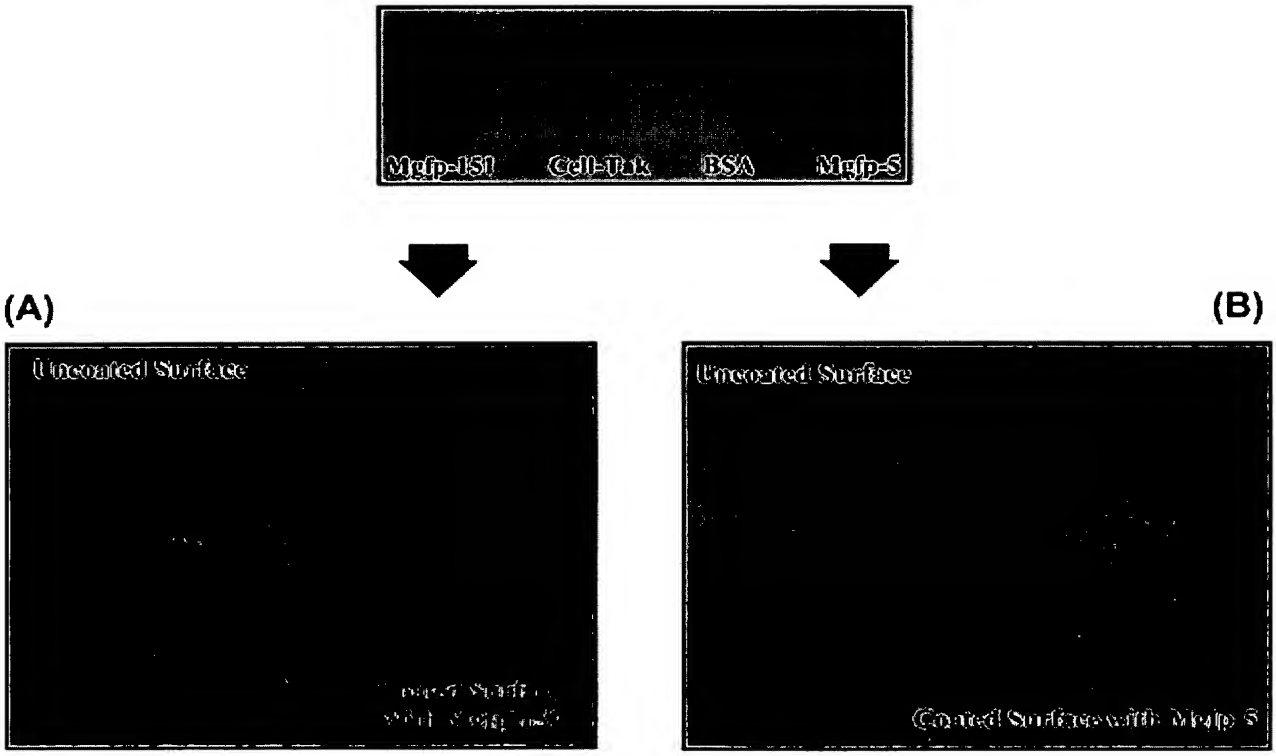
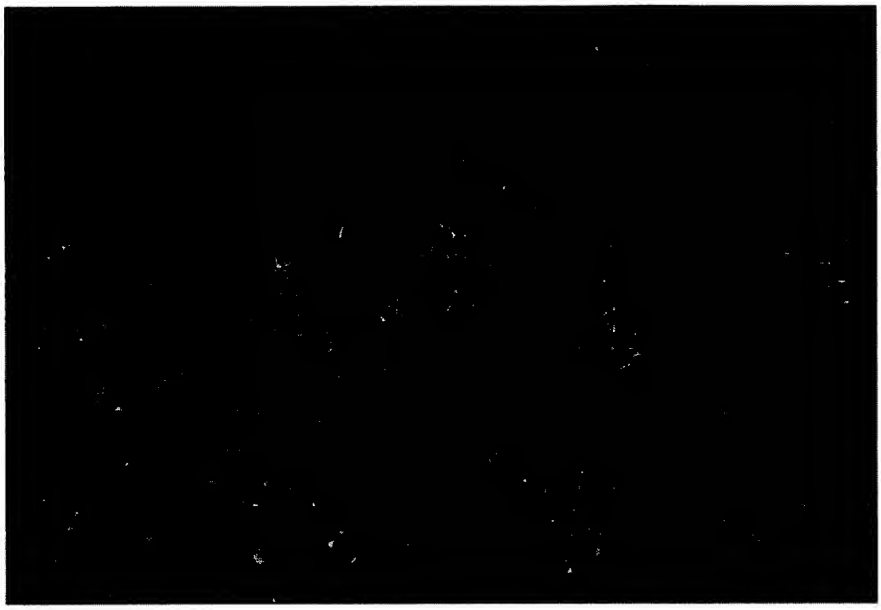

Mefp-1S1
Cell-Tak
BSA
Mefp-5
(A)
(B)
Uncoated Surface
Uncoated Surface
Coated Surface with Mefp-5

FIG.23

(A) Mgfp-151



(B) Mgfp-5

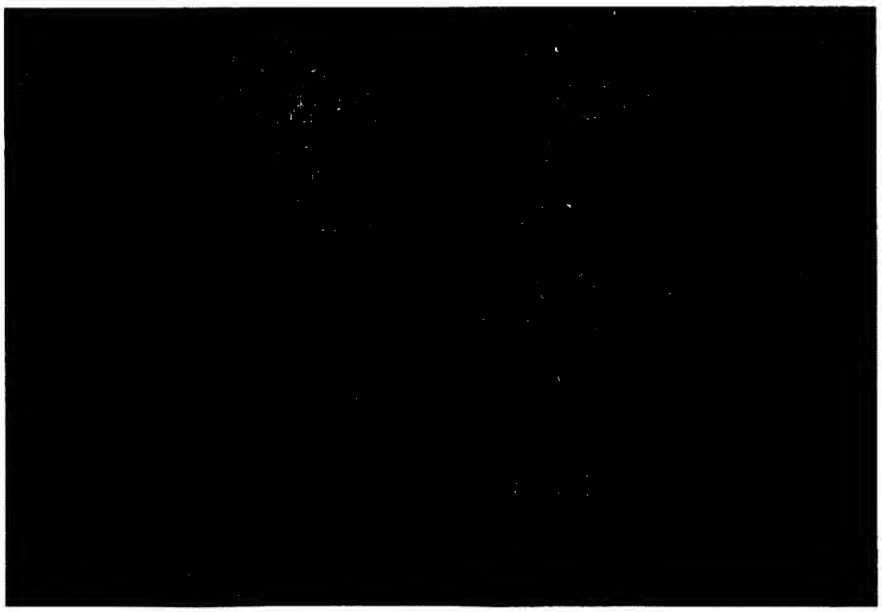


FIG. 24A

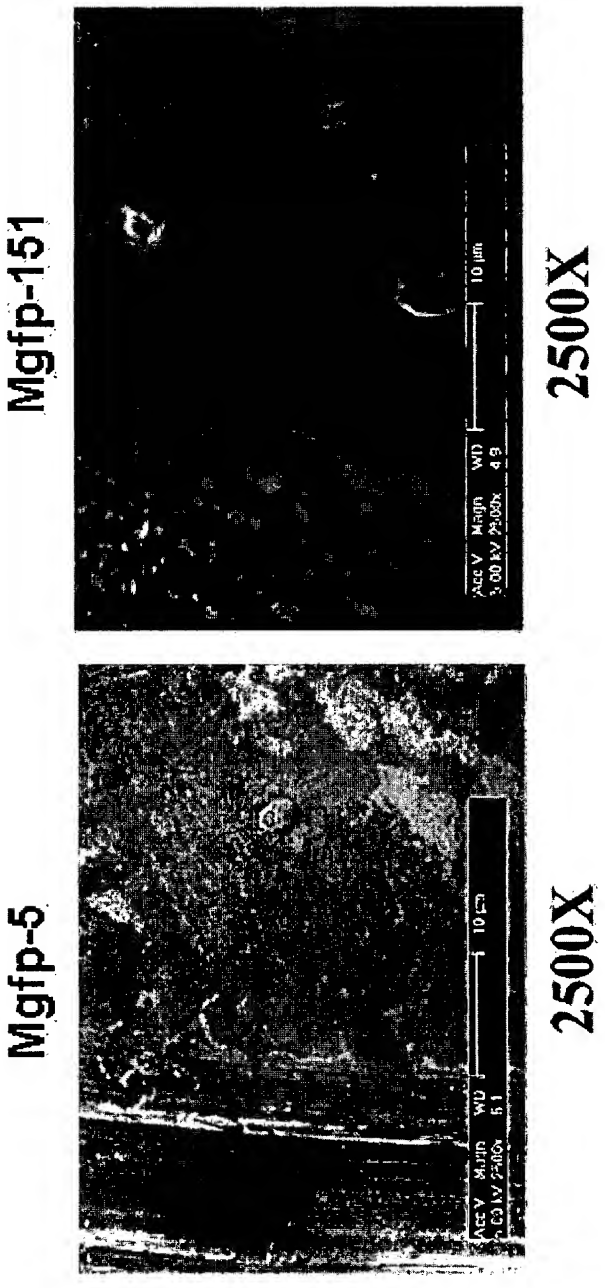


FIG. 24B

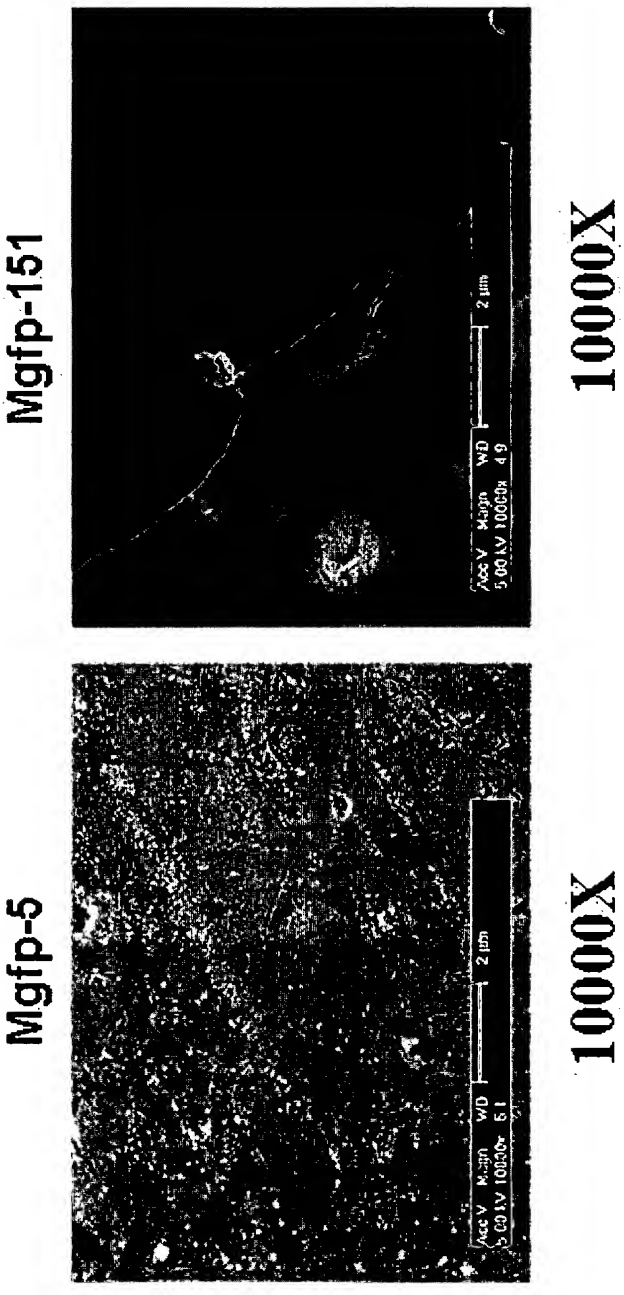
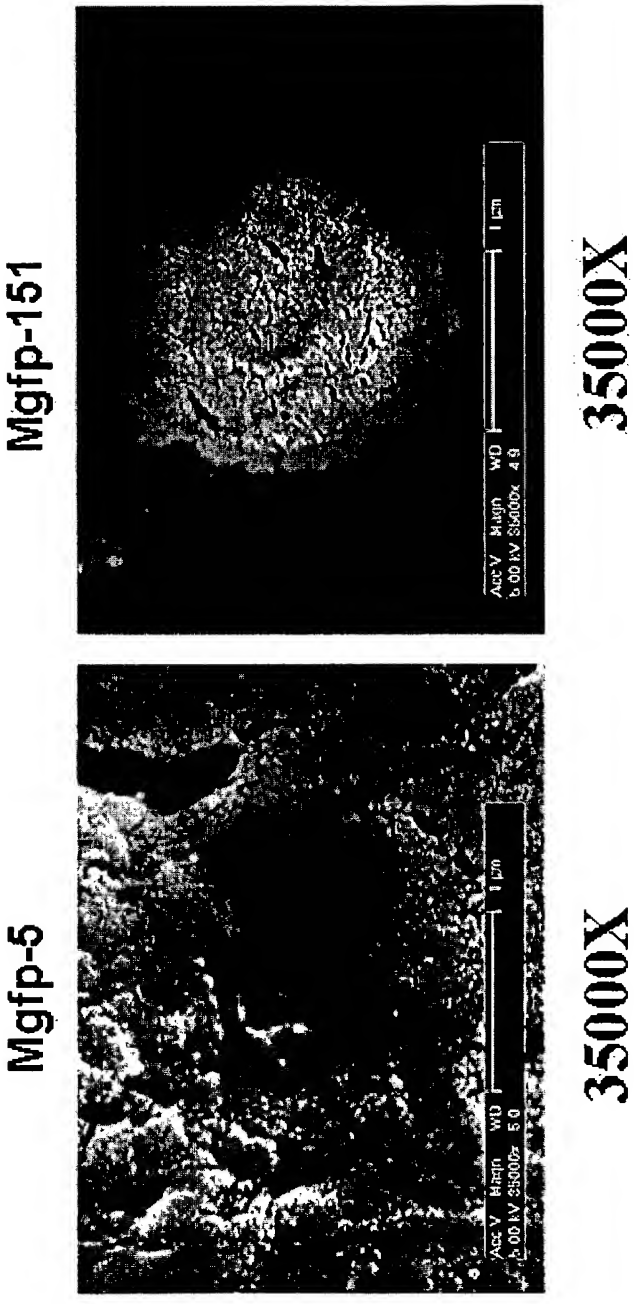


FIG. 24C



SEQUENCE LISTING

<110> POSCO
POSTECH Foundation

<120> Mussel Bioadhesive

<130> opp20050258kr

<150> US 60/556,805
<151> 2004-03-26

<160> 35

<170> KopatentIn 1.71

<210> 1
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 1
ggcctgcagc agttctgaag aatacaaggg 30

<210> 2
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 2
gtagatctat acgccggacc agtgaacag 29

<210> 3
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 3
cttgtatttt ccgctgtttt t 21

<210> 4
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 4
aaaaacagcg gaaaatacaa g 21

<210> 5
<211> 228
<212> DNA
<213> Mytilus galloprovincialis

<220>
<221> CDS
<222> (1)..(228)
<223> Mytilus galloprovincialis foot protein-5 cDNA

<400> 5
agt tct gaa gaa tac aaa ggt ggt tat tac cca ggc aat act tac cac 48
Ser Ser Glu Glu Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Thr Tyr His
1 5 10 15

tat cat tca ggt ggt agt tat cac gga tcc ggc tat cat gga gga tat 96
Tyr His Ser Gly Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr
20 25 30

aag gga aag tat tac gga aag gca aag aaa tac tat tat aaa tat aaa 144
Lys Gly Lys Tyr Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys
35 40 45

aac agc gga aaa tac aag tat ctg aag aaa gct aga aaa tac cat aga 192
Asn Ser Gly Lys Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg
50 55 60

aag ggt tac aag aag tat tat gga ggt ggt agc agt 228
Lys Gly Tyr Lys Lys Tyr Tyr Gly Gly Gly Ser Ser
65 70 75

<210> 6
<211> 76
<212> PRT
<213> Mytilus galloprovincialis

<400> 6
Ser Ser Glu Glu Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Thr Tyr His
1 5 10 15

Tyr His Ser Gly Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr
20 25 30

Lys Gly Lys Tyr Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys
35 40 45

Asn Ser Gly Lys Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg
50 55 60

Lys Gly Tyr Lys Lys Tyr Tyr Gly Gly Gly Ser Ser
65 70 75

<210> 7
<211> 180
<212> DNA
<213> mytilus edulis

<220>
<221> CDS
<222> (1)..(180)
<223> 6 times repeated sequence derived from mytilus edulis foot protein-1

<400> 7
gct aaa ccg tct tac ccg ccg acc tac aaa gca aaa ccc tcg tac cca 48
Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro
1 5 10 15

ccg act tat aag gct aaa cct agc tat cca cct acg tac aaa gct aaa 96
Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys
20 25 30

ccg tct tac ccg ccg act tac aaa gca aaa ccg tcc tac cct ccg acc 144
Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr
35 40 45

tat aag gct aaa ccg agt tac ccc ccg act tac aaa 180
Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys
50 55 60

<210> 8
<211> 60
<212> PRT
<213> mytilus edulis

<400> 8
Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro
1 5 10 15

Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys
20 25 30

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Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr
        35                  40                  45

Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys
    50                  55                  60


<210>   9
<211>   411
<212>   DNA
<213>   Artificial Sequence

<220>
<223>   Bioadhesive protein(mgfp-150) coding sequence


<220>
<221>   CDS
<222>   (1)..(411)
<223>   Bioadhesive protein(mgfp-150)


<400>   9
gct aaa ccg tct tac ccg ccg acc tac aaa gca aaa ccc tcg tac cca          48
Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro
  1               5                  10                  15

ccg act tat aag gct aaa cct agc tat cca cct acg tac aaa gct aaa          96
Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys
            20                  25                  30

ccg tct tac ccg ccg act tac aaa gca aaa ccg tcc tac cct ccg acc         144
Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr
        35                  40                  45

tat aag gct aaa ccg agt tac ccc ccg act tac aaa agt tct gaa gaa         192
Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ser Ser Glu Glu
    50                  55                  60

tac aag ggt ggt tat tac cca ggc aat tcg aac cac tat cat tca ggt         240
Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly
65                  70                  75                  80

ggt agt tat cac gga tcc ggc tac cat gga gga tat aag gga aag tat         288
Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr
                85                  90                  95

tac gga aag gca aag aaa tac tat tat aaa tat aaa aac agc gga aaa         336
Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys
            100                 105                 110

tac aag tat cta aag aaa gct aga aaa tac cat aga aag ggt tac aag         384
Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys
        115                 120                 125
```

aag tat tat gga ggt agc agt gaa ttc 411
Lys Tyr Tyr Gly Gly Ser Ser Glu Phe
130 135

<210> 10
<211> 137
<212> PRT
<213> Artificial Sequence

<400> 10
Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro
1 5 10 15

Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys
20 25 30

Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr
35 40 45

Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ser Ser Glu Glu
50 55 60

Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly
65 70 75 80

Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr
85 90 95

Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys
100 105 110

Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys
115 120 125

Lys Tyr Tyr Gly Gly Ser Ser Glu Phe
130 135

<210> 11
<211> 411
<212> DNA
<213> Artificial Sequence

<220>
<223> Bioadhesive protein(mgfp-051) coding sequence

<220>
<221> CDS
<222> (1)..(411)
<223> Bioadhesive protein(mgfp-051)

<400> 11

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agt tct gaa gaa tac aag ggt ggt tat tac cca ggc aat tcg aac cac          48
Ser Ser Glu Glu Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His
  1               5                  10                  15

tat cat tca ggt ggt agt tat cac gga tcc ggc tac cat gga gga tat          96
Tyr His Ser Gly Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr
             20                  25                  30

aag gga aag tat tac gga aag gca aag aaa tac tat tat aaa tat aaa         144
Lys Gly Lys Tyr Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys
         35                  40                  45

aac agc gga aaa tac aag tat cta aag aaa gct aga aaa tac cat aga         192
Asn Ser Gly Lys Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg
     50                  55                  60

aag ggt tac aag aag tat tat gga ggt agc agt gaa ttc gct aaa ccg         240
Lys Gly Tyr Lys Lys Tyr Tyr Gly Gly Ser Ser Glu Phe Ala Lys Pro
 65                  70                  75                  80

tct tac ccg ccg acc tac aaa gca aaa ccc tcg tac cca ccg act tat         288
Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr
                 85                  90                  95

aag gct aaa cct agc tat cca cct acg tac aaa gct aaa ccg tct tac         336
Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr
            100                 105                 110

ccg ccg act tac aaa gca aaa ccg tcc tac cct ccg acc tat aag gct         384
Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala
        115                 120                 125

aaa ccg agt tac ccc ccg act tac aaa                                     411
Lys Pro Ser Tyr Pro Pro Thr Tyr Lys
    130                 135
```

<210> 12
<211> 137
<212> PRT
<213> Artificial Sequence

<400> 12

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Ser Ser Glu Glu Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His
  1               5                  10                  15

Tyr His Ser Gly Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr
             20                  25                  30

Lys Gly Lys Tyr Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys
         35                  40                  45

Asn Ser Gly Lys Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg
     50                  55                  60
```

Lys Gly Tyr Lys Lys Tyr Tyr Gly Gly Ser Ser Glu Phe Ala Lys Pro
65 70 75 80

Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr
85 90 95

Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr
100 105 110

Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala
115 120 125

Lys Pro Ser Tyr Pro Pro Thr Tyr Lys
130 135

<210> 13
<211> 591
<212> DNA
<213> Artificial Sequence

<220>
<223> Bioadhesive protein(mgfp-151) coding sequence

<220>
<221> CDS
<222> (1)..(591)
<223> Bioadhesive protein(mgfp-151)

<400> 13
gct aaa ccg tct tac ccg ccg acc tac aaa gca aaa ccc tcg tac cca 48
Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro
1 5 10 15

ccg act tat aag gct aaa cct agc tat cca cct acg tac aaa gct aaa 96
Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys
20 25 30

ccg tct tac ccg ccg act tac aaa gca aaa ccg tcc tac cct ccg acc 144
Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr
35 40 45

tat aag gct aaa ccg agt tac ccc ccg act tac aaa agt tct gaa gaa 192
Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ser Ser Glu Glu
50 55 60

tac aag ggt ggt tat tac cca ggc aat tcg aac cac tat cat tca ggt 240
Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly
65 70 75 80

ggt agt tat cac gga tcc ggc tac cat gga gga tat aag gga aag tat 288
Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr

85 90 95

tac gga aag gca aag aaa tac tat tat aaa tat aaa aac agc gga aaa 336
Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys
100 105 110

tac aag tat cta aag aaa gct aga aaa tac cat aga aag ggt tac aag 384
Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys
115 120 125

aag tat tat gga ggt agc agt gaa ttc gct aaa ccg tct tac ccg ccg 432
Lys Tyr Tyr Gly Gly Ser Ser Glu Phe Ala Lys Pro Ser Tyr Pro Pro
130 135 140

acc tac aaa gca aaa ccc tcg tac cca ccg act tat aag gct aaa cct 480
Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro
145 150 155 160

agc tat cca cct acg tac aaa gct aaa ccg tct tac ccg ccg act tac 528
Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr
165 170 175

aaa gca aaa ccg tcc tac cct ccg acc tat aag gct aaa ccg agt tac 576
Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr
180 185 190

ccc ccg act tac aaa 591
Pro Pro Thr Tyr Lys
195

<210> 14
<211> 197
<212> PRT
<213> Artificial Sequence

<400> 14
Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro
1 5 10 15

Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys
20 25 30

Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr
35 40 45

Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ser Ser Glu Glu
50 55 60

Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly
65 70 75 80

Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr
85 90 95

Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys
100 105 110

Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys
115 120 125

Lys Tyr Tyr Gly Gly Ser Ser Glu Phe Ala Lys Pro Ser Tyr Pro Pro
130 135 140

Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro
145 150 155 160

Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr
165 170 175

Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr
180 185 190

Pro Pro Thr Tyr Lys
195

<210> 15
<211> 354
<212> DNA
<213> Artificial Sequence

<220>
<223> construct for expression of Bioadhesive protein(mgfp-5) in pMDG05 vector

<220>
<221> CDS
<222> (1)..(351)
<223> Bioadhesive recombinant protein expressed in pMDG05 vector

<400> 15
atg ggg ggt tct cat cat cat cat cat cat ggt atg gct agc atg act 48
Met Gly Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

ggt gga cag caa atg ggt cgg act ctg tac gac gat gac gat aag gat 96
Gly Gly Gln Gln Met Gly Arg Thr Leu Tyr Asp Asp Asp Asp Lys Asp
20 25 30

cga tgg gga tcc gag ctc gag atc tgc agc agt tct gaa gaa tac aag 144
Arg Trp Gly Ser Glu Leu Glu Ile Cys Ser Ser Ser Glu Glu Tyr Lys
35 40 45

ggt ggt tat tac cca ggc aat tcg aac cac tat cat tca ggt ggt agt 192
Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly Gly Ser
50 55 60

tat cac gga tcc ggc tac cat gga gga tat aag gga aag tat tac gga 240
Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr Tyr Gly
65 70 75 80

aag gca aag aaa tac tat tat aaa tat aaa aac agc gga aaa tac aag 288
Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys Tyr Lys
85 90 95

tat cta aag aaa gct aga aaa tac cat aga aag ggt tac aag aag tat 336
Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys Lys Tyr
100 105 110

tat gga ggt agc agt taa 354
Tyr Gly Gly Ser Ser
115

<210> 16
<211> 117
<212> PRT
<213> Artificial Sequence

<400> 16
Met Gly Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Thr Leu Tyr Asp Asp Asp Asp Lys Asp
20 25 30

Arg Trp Gly Ser Glu Leu Glu Ile Cys Ser Ser Ser Glu Glu Tyr Lys
35 40 45

Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly Gly Ser
50 55 60

Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr Tyr Gly
65 70 75 80

Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys Tyr Lys
85 90 95

Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys Lys Tyr
100 105 110

Tyr Gly Gly Ser Ser
115

<210> 17
<211> 456
<212> DNA
<213> Artificial Sequence

<220>
<223> construct for expression of Bioadhesive protein(mgfp-150) in

pMDG150 vector

<220>
<221> CDS
<222> (1)..(453)
<223> Bioadhesive recombinant protein expressed in pMDG150 vector

<400> 17

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atg ggg ggt tct cat cat cat cat cat cat ggt atg gct agc gct aaa          48
Met Gly Gly Ser His His His His His His Gly Met Ala Ser Ala Lys
  1               5                  10                  15

ccg tct tac ccg ccg acc tac aaa gca aaa ccc tcg tac cca ccg act          96
Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr
             20                  25                  30

tat aag gct aaa cct agc tat cca cct acg tac aaa gct aaa ccg tct         144
Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser
         35                  40                  45

tac ccg ccg act tac aaa gca aaa ccg tcc tac cct ccg acc tat aag         192
Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys
     50                  55                  60

gct aaa ccg agt tac ccc ccg act tac aaa ggc tgc agt tct gaa gaa         240
Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Gly Cys Ser Ser Glu Glu
 65                  70                  75                  80

tac aag ggt ggt tat tac cca ggc aat tcg aac cac tat cat tca ggt         288
Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly
                 85                  90                  95

ggt agt tat cac gga tcc ggc tac cat gga gga tat aag gga aag tat         336
Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr
            100                 105                 110

tac gga aag gca aag aaa tac tat tat aaa tat aaa aac agc gga aaa         384
Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys
        115                 120                 125

tac aag tat cta aag aaa gct aga aaa tac cat aga aag ggt tac aag         432
Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys
    130                 135                 140

aag tat tat gga ggt agc agt         taa                                 456
Lys Tyr Tyr Gly Gly Ser Ser
145                 150
```

<210> 18
<211> 151
<212> PRT
<213> Artificial Sequence

<400> 18
Met Gly Gly Ser His His His His His His Gly Met Ala Ser Ala Lys
1 5 10 15

Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr
20 25 30

Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser
35 40 45

Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys
50 55 60

Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Gly Cys Ser Ser Glu Glu
65 70 75 80

Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly
85 90 95

Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr
100 105 110

Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys
115 120 125

Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys
130 135 140

Lys Tyr Tyr Gly Gly Ser Ser
145 150

<210> 19
<211> 540
<212> DNA
<213> Artificial Sequence

<220>
<223> construct for expression of Bioadhesive protein(mgfp-051) in pMDG051 vector

<220>
<221> CDS
<222> (1)..(537)
<223> Bioadhesive recombinant protein expressed in pMDG051 vector

<400> 19
atg ggg ggt tct cat cat cat cat cat cat ggt atg gct agc atg act 48
Met Gly Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

ggt gga cag caa atg ggt cgg act ctg tac gac gat gac gat aag gat 96

```
Gly Gly Gln Gln Met Gly Arg Thr Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

cga tgg gga tcc gag ctc gag atc tgc agc agt tct gaa gaa tac aag      144
Arg Trp Gly Ser Glu Leu Glu Ile Cys Ser Ser Ser Glu Glu Tyr Lys
        35                  40                  45

ggt ggt tat tac cca ggc aat tcg aac cac tat cat tca ggt ggt agt      192
Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly Gly Ser
    50                  55                  60

tat cac gga tcc ggc tac cat gga gga tat aag gga aag tat tac gga      240
Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr Tyr Gly
65                  70                  75                  80

aag gca aag aaa tac tat tat aaa tat aaa aac agc gga aaa tac aag      288
Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys Tyr Lys
                85                  90                  95

tat cta aag aaa gct aga aaa tac cat aga aag ggt tac aag aag tat      336
Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys Lys Tyr
            100                 105                 110

tat gga ggt agc agt gaa ttc gct aaa ccg tct tac ccg ccg acc tac      384
Tyr Gly Gly Ser Ser Glu Phe Ala Lys Pro Ser Tyr Pro Pro Thr Tyr
        115                 120                 125

aaa gca aaa ccc tcg tac cca ccg act tat aag gct aaa cct agc tat      432
Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr
    130                 135                 140

cca cct acg tac aaa gct aaa ccg tct tac ccg ccg act tac aaa gca      480
Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala
145                 150                 155                 160

aaa ccg tcc tac cct ccg acc tat aag gct aaa ccg agt tac ccc ccg      528
Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro
                165                 170                 175

act tac aaa       taa       540
Thr Tyr Lys

<210> 20
<211> 179
<212> PRT
<213> Artificial Sequence

<400> 20
Met Gly Gly Ser His His His His His His Gly Met Ala Ser Met Thr
  1               5                  10                  15

Gly Gly Gln Gln Met Gly Arg Thr Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30
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Arg Trp Gly Ser Glu Leu Glu Ile Cys Ser Ser Ser Glu Glu Tyr Lys
35 40 45

Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly Gly Ser
50 55 60

Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr Tyr Gly
65 70 75 80

Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys Tyr Lys
85 90 95

Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys Lys Tyr
100 105 110

Tyr Gly Gly Ser Ser Glu Phe Ala Lys Pro Ser Tyr Pro Pro Thr Tyr
115 120 125

Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr
130 135 140

Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala
145 150 155 160

Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro
165 170 175

Thr Tyr Lys

<210> 21
<211> 642
<212> DNA
<213> Artificial Sequence

<220>
<223> construct for expression of Bioadhesive protein(mgfp-151) in pMDG151 vector

<220>
<221> CDS
<222> (1)..(639)
<223> Bioadhesive recombinant protein expressed in pMDG151 vector

<400> 21
atg ggg ggt tct cat cat cat cat cat cat ggt atg gct agc gct aaa 48
Met Gly Gly Ser His His His His His His Gly Met Ala Ser Ala Lys
1 5 10 15

ccg tct tac ccg ccg acc tac aaa gca aaa ccc tcg tac cca ccg act 96
Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr
20 25 30

```
tat aag gct aaa cct agc tat cca cct acg tac aaa gct aaa ccg tct          144
Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser
          35                  40                  45

tac ccg ccg act tac aaa gca aaa ccg tcc tac cct ccg acc tat aag          192
Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys
     50                  55                  60

gct aaa ccg agt tac ccc ccg act tac aaa ggc tgc agt tct gaa gaa          240
Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Gly Cys Ser Ser Glu Glu
 65                  70                  75                  80

tac aag ggt ggt tat tac cca ggc aat tcg aac cac tat cat tca ggt          288
Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly
                 85                  90                  95

ggt agt tat cac gga tcc ggc tac cat gga gga tat aag gga aag tat          336
Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr
            100                 105                 110

tac gga aag gca aag aaa tac tat tat aaa tat aaa aac agc gga aaa          384
Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys
        115                 120                 125

tac aag tat cta aag aaa gct aga aaa tac cat aga aag ggt tac aag          432
Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys
    130                 135                 140

aag tat tat gga ggt agc agt gaa ttc gct aaa ccg tct tac ccg ccg          480
Lys Tyr Tyr Gly Gly Ser Ser Glu Phe Ala Lys Pro Ser Tyr Pro Pro
145                 150                 155                 160

acc tac aaa gca aaa ccc tcg tac cca ccg act tat aag gct aaa cct          528
Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro
                165                 170                 175

agc tat cca cct acg tac aaa gct aaa ccg tct tac ccg ccg act tac          576
Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr
            180                 185                 190

aaa gca aaa ccg tcc tac cct ccg acc tat aag gct aaa ccg agt tac          624
Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr
        195                 200                 205

ccc ccg act tac aaa t aa                                                642
Pro Pro Thr Tyr Lys
    210
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<210> 22
<211> 213
<212> PRT
<213> Artificial Sequence

<400> 22

Met Gly Gly Ser His His His His His His Gly Met Ala Ser Ala Lys
1 5 10 15

Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr
20 25 30

Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser
35 40 45

Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys
50 55 60

Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Gly Cys Ser Ser Glu Glu
65 70 75 80

Tyr Lys Gly Gly Tyr Tyr Pro Gly Asn Ser Asn His Tyr His Ser Gly
85 90 95

Gly Ser Tyr His Gly Ser Gly Tyr His Gly Gly Tyr Lys Gly Lys Tyr
100 105 110

Tyr Gly Lys Ala Lys Lys Tyr Tyr Tyr Lys Tyr Lys Asn Ser Gly Lys
115 120 125

Tyr Lys Tyr Leu Lys Lys Ala Arg Lys Tyr His Arg Lys Gly Tyr Lys
130 135 140

Lys Tyr Tyr Gly Gly Ser Ser Glu Phe Ala Lys Pro Ser Tyr Pro Pro
145 150 155 160

Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro
165 170 175

Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr
180 185 190

Lys Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys Ala Lys Pro Ser Tyr
195 200 205

Pro Pro Thr Tyr Lys
210

<210> 23
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 23
ggtacccgaa ttcgaattcg ctaaaccg 28

<210> 24
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 24
ggtcgactca agcttatcat ttgtaagtcg 30

<210> 25
<211> 10
<212> PRT
<213> mytilus edulis

<400> 25
Ala Lys Pro Ser Tyr Pro Pro Thr Tyr Lys
1 5 10

<210> 26
<211> 30
<212> DNA
<213> Mytilus edulis

<400> 26
gctaaaccgt cttacccgcc gacctacaaa 30

<210> 27
<211> 30
<212> DNA
<213> Mytilus edulis

<400> 27
gcaaaaccct cgtacccacc gacttataag 30

<210> 28
<211> 30
<212> DNA
<213> Mytilus edulis

<400> 28
gctaaaccta gctatccacc tacgtacaaa 30

<210> 29
<211> 30
<212> DNA

<213> Mytilus edulis

<400> 29
gctaaaccgt cttacccgcc gacttacaaa 30

<210> 30
<211> 30
<212> DNA
<213> Mytilus edulis

<400> 30
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2005/000888

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07K 14/435, C12N 15/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7: C07K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
PubMed, NCBI, Delphion, eKIPASS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	NCBI Accession No. AAS00463 (Feb. 01, 2004)	1, 10 2-9, 11-49
A	US 5202236 (Enzon Labs Inc., Apr. 13, 1993), See entire document.	2-9, 11-49
A	JP 8-266281 (Kaiyo Biotechnol Kenkyusho:KK., Oct. 15, 1996), See entire document.	2-9, 11-49
A	Deming, T.J. "Mussel byssus and biomolecular materials" Vol. 3: pages 100-105 (1999), See entire document.	2-9, 11-49

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 JULY 2005 (21.07.2005)	**22 JULY 2005 (22.07.2005)**

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	PARK, JEONG UNG Telephone No. 82-42-481-8159

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2005/000888

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of :

   a. type of material
   - [x] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material
   - [x] in written format
   - [x] in computer readable form

   c. time of filing/furnishing
   - [x] contained in the international application as filed
   - [x] filed together with the international application in computer readable form
   - [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2005/000888

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5202236	Apr. 13, 1993	EP 0304486 A1	Mar. 01, 1989
		WO 8807076 A1	Sep. 22, 1988